FLUCTUATING ASYMMETRY AND BODY MORPHOLOGY IN RELATION TO POPULATION BOTTLENECKS OF INTRODUCED BIRDS IN NEW ZEALAND

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GENERAL ABSTRACT

The introduction of exotic bird species to New Zealand (NZ) from the United Kingdom (UK) over 100 years ago unintentionally created an ideal study system to examine potential changes in developmental stability due to bottleneck effects. In this study I measured fluctuating asymmetry (FA; random deviations from symmetry between bilaterally symmetrical traits) in 13 species of introduced birds in NZ. FA has been used for conservation purposes as an early warning system of increased developmental instability (DI; the inability to cope with random genetic or environmental perturbations during development). I evaluated DI using FA in several anatomical external and internal morphological traits, and compared differences in body morphology between introduced and source populations in relation to the bottleneck size. I also examined FA in nestlings in two closely related introduced species that passed through two different-sized population bottlenecks. Differences in FA in relation to bottleneck severity were only observed in external traits. FA in external traits in some NZ populations differed from their UK counterparts, but it was in the opposite direction than predicted. FA in external traits varied significantly across NZ populations of introduced species – the most severe bottlenecks species exhibited higher levels of FA than species that passed through larger bottlenecks. There were no patterns in FA and bottleneck size for skeletal traits, most likely due to differences in environmental and genetic stressors resulting in species- and character-specific FA relationships. Nestling FA was the same for both species, despite the large difference in bottleneck size. FA did decrease over the nestling period, although not at the same rate for each trait, most likely due to the differing costs of development, functional importance, and other environmental stressors that might influence FA in each trait differently. Overall changes in body morphology occurred in four species introduced to NZ, and all species exhibited some changes in trait morphology but were not related to bottleneck size. Finally, the proportion of deformities (deviations from normal phenotype) was higher in NZ than in UK suggesting passing through a bottleneck increased the probability of abnormalities. Although the associations between FA, body morphology and bottleneck severity are complex, my results confirm that measures of morphology have the potential of being useful indicators of DI in the management of endangered species.

PREFACE TO THE DISSERTATION

As a post-graduate student, I agreed to submit for publication all chapters of this thesis as they were completed. Since each manuscript has to stand alone, I am required to include some duplicate explanatory information in some sections of certain chapters.

Below is a list of chapters, as they will be published.

Chapter 1: Fluctuating asymmetry as an indicator of developmental instability in introduced species in New Zealand.

Chapter 2: Fluctuating asymmetry in the skeletal structure of introduced birds in New Zealand.

Chapter 3: Fluctuating asymmetry in the nestlings of Common Mynas and European Starlings introduced to New Zealand.

Chapter 4: Comparative morphometrics of introduced birds in New Zealand.

Chapter 5: Population bottlenecks and physical abnormalities in introduced birds of New Zealand.

GENERAL INTRODUCTION

Inbreeding depression is defined as a decrease in the fitness of a trait as a direct consequence of mating with closely related individuals (Wright 1977; Shields 1987; Crnokrak and Roff 1999). This may lead to an increase in developmental problems such as an increase in developmental instability (DI), which is due to the inability of an individual to cope with random genetic or environmental perturbations during development (Parsons 1992; Palmer and Strobeck 1992; Lens et al. 2002a). For example, Lens et al. (2000) found higher levels of inbreeding and DI in critically endangered Taita Thrushes (*Turdus helleri*) found in habitats with high levels of disturbance. Moreover, Swaddle and Witter (1994) found that both nutritional and energetic stress increased DI in European Starlings (*Sturnus vulgaris*). DI can be observed physically in an individual, and thus quantified, through the measurement of deviations from symmetry in bilaterally symmetrical morphometric traits. Asymmetry due to abnormal deviations is known as fluctuating asymmetry (FA; Van Valen 1962; Palmer and Strobeck 1986).

The level of FA has been widely used as a measurement index of an individual's fitness or assess the individual's inability to cope with stress, however determining which traits should be used to measure FA is controversial. Rintamäki et al. (1997) found that tarsus asymmetry in male Black Grouse (*Tetrao tetrix*) led to a decrease in copulation success and suggested that this trait may reflect male quality. On the other hand, Gilligan et al. (1999) found inconsistent relationships between FA of bristle numbers and inbreeding in fruit flies (*Drosophila melanogaster*). Karvonen et al. (2003) also noted inconsistencies in levels of FA across various traits between two populations of Greenfinches (*Carduelis chloris*) of differing genetic diversity. A

further problem is that most studies using FA to study stress have generally been limited to a single season or age of the individual, which may not be representative if DI is expressed at differing levels in different morphological traits during different stages of an individual's lifetime (Berggren 2005). Thus, understanding FA patterns, and determining which trait, or combination of traits, can be most consistently and reliably used to measure FA, could have important applications in a wide variety of fields, including conservation biology in which it is important to have robust indices of animal health and stress (Lens et al. 2002a). It is also important to note that FA is not necessarily maladaptive. Many species have asymmetrical morphological traits that have evolved for survival (e.g., ear asymmetry in owls, Norberg 1977). However, if one is hypothesizing that FA in traits may be pre-adaptations for future evolution of DA, the asymmetry would have to be beneficial throughout all stages of evolution. As most of the traits examined in this study have been chosen due to their functional importance in a symmetric state, I have assumed that observed asymmetries (i.e., asymmetrical wings) would have negative fitness consequences (see Swaddle 1997).

The accuracy of measuring FA has increased tremendously since it was first suggested as a tool to evaluate DI. Palmer and Strobeck (1986) developed and detailed over ten FA indices that could be used. These have since been refined to further improve their accuracy (see Palmer 1994, Palmer and Strobeck 2003). Throughout this study, I used 4 different estimates of FA: FA1 (the mean absolute value of the mean right minus left measurements), FA4a (the magnitude of FA including ME), FA10a (the magnitude of FA as a proportion of the trait mean after removing ME), and FA8a (the size-scaled estimate of between-sides variance). Calculating FA involves a ten-step procedure using a series of scatterplots from the

combination of traits chosen to measure FA, followed by Grubb's Tests (Sokal and Rohlf 1995) to remove outliers and any anomalous individuals (Palmer and Strobeck 2003). To evaluate if measurement error (ME) is negligible compared to FA, two-way mixed model ANOVAs using sequential Bonferoni corrections (Sokal and Rohlf 1995) for every trait and group are conducted separately using the measurements of each side (as a fixed factor), and individuals (as a random factor). Lastly, skew and kurtosis of the frequency distributions of the between-sides measurements along with three-way ANOVAs for every trait examined are performed to test for the presence of Directional Asymmetry (DA) and antisymmetry in relation to FA.

FA1 is one of the most common FA indices used in biological studies (Palmer and Strobeck 2003). According to Palmer (1994), FA1 is frequently used as a general index of FA because it can be easily calculated, however it may not account for the presence of DA or antisymmetry and may be dependent on trait size. As a result, it is recommended to use several FA indices to verify the robustness of FA results. FA4a is more sensitive to outliers than FA1 but is highly sensitive to DA, therefore it is often used to check for the presence of DA in conjunction with FA1. In addition, FA10a is also used in conjunction with FA1 as it is one of only two FA indices that describes the between sides variance once ME has been removed. However, FA10a cannot stand alone as a reliable FA index as it can be biased in the presence of DA (Palmer 1994, Palmer and Strobeck 2003). More recent studies have also suggested that FA may be a more reliable indicator of DI if evaluated across a combination of traits (e.g., Lens et al. 2002). FA8a allows for comparison of FA across different traits and/or species by correcting for size, which may allow for an overall picture of FA patterns across individuals and populations.

The measurement of FA might be an especially useful tool in the management of endangered species that have passed through a severe population bottleneck. A bottleneck is simply the decrease of a population to a low number of individuals. Even if such a population recovers through conservation initiatives, bottlenecks can cause a long-term depletion in an individual's genetic variability by lowering heterozygosity and increasing the expression of deleterious genes in future generations (Frankham et al. 2002). This in turn may lead to a decrease in fitness that can be observed through changes in various traits (e.g., an increase in rate of hatching failure, Briskie and Mackintosh 2004), including an increase in the levels of FA.

Although a population that passes through a severe bottleneck may manage to successfully establish itself in a new location, it is not always obvious that such a population experiences negative fitness consequences as a result. Morphological traits (either external or internal anatomical traits) can also change after a population has passed through a bottleneck (e.g., Amiot et al. 2007). Moreover, some of these changes may adapt a population to its new surroundings or in extreme cases changes in body morphology may result in physical deformities that could have detrimental effects on survival. As body morphology is a common indicator of fitness in many avian studies (e.g., Brown et al. 1993; Murphy 2007; Hedenström 2008), changes in body morphology may also reveal important biological changes a population may be experiencing. Thus, exploring both physical changes in appearance as well as changes in the levels of FA may be useful methods of evaluating the fitness consequences of a bottleneck. The development of such techniques would also serve as a useful tool in future translocation and re-introduction programs of endangered species in which it is possible to select bottleneck sizes that do not jeopardize the long-term viability of the translocated population.

Even though the use of FA and other morphological traits might be useful techniques for the management of endangered wildlife, it is particularly difficult to study such species, both because of their rarity and because there is often no large and non-bottlenecked populations that can be used as a control for comparative purposes. However, as with other places around the world, the settlement of New Zealand (NZ) led to the introduction of many exotic plant and animal species. Many species of birds were introduced in New Zealand in the 19th century as part of various acclimatization programs (Lever 1987). For example, House Sparrow (*Passer domesticus*), European Starling, Blackbird (*Turdus merula*), Song Thrush (*T. philomelos*), Chaffinch (*Fringella coelebs*), Greenfinch, Goldfinch (*Carduelis carduelis*), Redpoll (*Carduelis flammea*), Yellowhammer (*Emberiza citronella*), Cirl Bunting (*E. cirlus*), Dunnock (*Prunella modularis*), Skylark (*Alauda arvensis*), and Rook (*Corvus frugilegus*) were introduced from the UK. Other species were also introduced from other parts of the world: Common Myna (*Acridotheres tristis*) and Australian Magpie (*Gymnorhina tibicen*) populations were brought from Australia, while Canada Geese (*Branta canadensis maxima*) and Californian Quails (*Callipepla californica brunnescens*) were brought in from Canada and the US, respectively (Lever 1987).

As with native endangered birds, each of these introduced species passed through a bottleneck during their establishment. However, in this case the size of the bottleneck was the number of individuals released, and it varied across species from as few as 11 birds in the Cirl Bunting to 60 birds in the Canada Goose, 100 birds in the Greenfinch, 250 birds in the Dunnock, 500 in the Chaffinch, 800 in the Blackbird, and 1100 birds in the California Quail (Lever 1987). These numbers accounted only for populations that were reported to be successful. In addition, introductions

occurred over several years in various locations across New Zealand, and the age and sex ratios of the populations released were unknown. Nevertheless, records for the overall numbers of birds released, along with their release dates, are fairly accurate due to the extensive documentation by the acclimatization societies responsible for their importation (Thomson 1922). This makes the use of introduced species ideal for study of FA since the severity of the bottleneck is known for each species from the number of birds that founded a given population. Moreover, this study system allows for the comparison of established populations in NZ, with the source populations in their native range. This is something that is not possible with native endangered birds as many such species no longer have "non-bottlenecked" populations to use as controls (Briskie 2006).

Throughout this study, I assume that the differences in bottleneck size between my study species have lead to differences in their current level of genetic variation present. This may not be the case as population bottlenecks do not necessarily have strong effects on genetic variability if there is a rapid increase in population size after the bottleneck event (Frankham et al. 2002). Moreover, previous genetic analyses on introduced species in NZ have shown little or no evidence of changes in average expected heterozygosity (e.g., Ross 1983; Parkin and Cole 1985; Baker and Moeed 1987; Baker 1992; Merila et al. 1996), however improvements in genetic techniques have since been made and these earlier studies need to be repeated. This further stresses the need for additional genetic analyses to quantify the relationship between FA and inbreeding – and to determine if the remaining species of introduced birds to NZ populations display signs of genetic drift or decreased genetic variability. However, since these studies have not been undertaken, my examination of the relationship between population bottleneck effects and changes in FA and body

morphology is necessarily based on the assumption that more severe bottlenecks are more likely to lead to deleterious fitness consequences. This assumption appears justified given the finding that some species of introduced birds show problems with hatching success despite passing through what might be considered relatively large bottlenecks (Briskie and Mackintosh 2004).

In this study I examined changes in a number of physical attributes of introduced birds in NZ by using FA, comparative morphological measurements, and visual observations of abnormalities in both the post-bottlenecked populations and in the non-bottlenecked source populations. My objective was to determine whether changes in FA and morphology might have potentially been a result of the size of the bottleneck each species passed through during their establishment. The results of my study should provide a better understanding of FA as an indicator of DI in introduced, and potentially inbred species, and the potential outcomes in physical changes as a result of population bottlenecks, which may aid in conservation management programs of endangered species.

Outline of thesis

In chapter 1, I examined the patterns of FA in a variety of external features such as wing length, bill length and tarsus length, and the length of the outermost primary feather (P9). I tested the hypothesis that the levels of FA in relation to bottleneck size will differ, such that within-species FA in external traits will be greater in introduced (bottlenecked) populations than their source populations, and the level of FA in traits should decrease as the size of the bottleneck increases. I also explored these concepts in chapter 2 using osteological characters, as they may be a useful index of long-term stresses an individual may be experiencing because bone

growth is likely influenced during embryonic and nestling development (Maul and Farris 2005). Since levels of FA have also been known to vary throughout the course of an individual's lifetime (Berggren 2005), and random perturbations could affect traits such as feathers at both the embryonic stage of development and during growth (Stige et al. 2005), I next examined patterns of FA in nestlings during the nestling period of two closely related introduced species – the Starling and the Myna – that have passed through different-sized population bottlenecks during their establishment. These results are presented in chapter 3. Population bottlenecks and introductions to new habitats could also result in a change in body morphology, thus in chapter 4, I compared differences in trait and body morphology between introduced NZ populations and their source populations in the UK. In chapter 5, I examined the frequency of changes in morphology that resulted in physical abnormalities. Lastly, in my general discussion I reviewed the implications of my results, their applications to conservation management, and possible future work that could be performed to further improve the reliability of my findings as indicators of DI in bottlenecked populations.

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CHAPTER 1: Fluctuating asymmetry as an indicator of developmental instability in introduced birds in New Zealand

Abstract

Fluctuating asymmetry (FA; random deviations from symmetry between bilaterally symmetrical traits) has become a common index to evaluate developmental instability. In this study I examined levels of FA in four external morphological traits among ten species of passerines that were introduced to New Zealand (NZ). I compared levels of FA between the introduced and source populations in relation to the severity of the bottleneck each passed through during their establishment. I tested the hypothesis that levels of FA should differ in relation to bottleneck size, such that within-species FA should be greater in introduced (bottlenecked) populations than source populations, and that the level of FA should decrease as the size of the bottleneck increased. Within-species comparisons revealed that the FA of the tarsus length of Redpolls (*Carduelis flammea*) was higher in the UK population, opposite to that expected. In contrast, comparisons across species within NZ revealed that the FA of the wing chord in hatch year individuals and the FA of the ninth primary in hatch-year females were significantly greater in populations that passed through the most severe bottlenecks. FA in NZ populations, compared to UK populations, also differed for the bill length of hatch year males in relation to bottleneck size, but it was in the opposite direction than predicted with UK populations of some species exhibiting higher FA than their NZ counterparts. Although I found the association between FA and bottleneck severity was likely confounded by a variety of environmental variables that make it difficult to compare between locations, it has the potential of being a useful indicator of developmental instability in conservation management when used with other measures of fitness and with an understanding of levels of FA in non-endangered populations.

Introduction

Translocation of organisms by humans from one place to another, whether for species conservation, pest and habitat management, aesthetic purposes, or accidentally, has been a common occurrence over the past century (e.g., Griffith et al. 1989). The primary concern regarding translocations for species conservation is survival and stability of the introduced population at its new location. Most introduced populations pass through a population bottleneck (a decrease in number of individuals from the original population size) that can have detrimental effects on individuals by lowering heterozygosity, increasing expression of deleterious alleles in future generations, and by increasing the likelihood of inbreeding (Nei et al. 1974; Frankham et al. 2002). Furthermore, inbreeding depression (a decrease in fitness of a population due to inbreeding; Wright 1977; Shields 1987; Crnokrak and Roff 1999) can lead to an increase in developmental instability, which is the inability to cope with random genetic or environmental perturbations during development (Parsons 1992; Palmer and Strobeck 1992; Lens et al. 2002) of individuals of a population that has passed through a bottleneck.

Fluctuating asymmetry (FA) – random deviations from symmetry between a bilaterally symmetrical trait (Van Valen 1962; Palmer and Strobeck 1986) – has become a commonly used measure of developmental instability (DI). Maul and Farris (2005) demonstrated that FA might be useful in assessing environmental stressors in Northern Cardinals (*Cardinalis cardinalis)* inhabiting pesticide-treated agricultural areas. Similarly, Anciaes and Marini (2000) found higher levels of FA in passerine birds inhabiting fragmented areas of Brazilian tropical forests. FA has also been used in a variety of studies to assess fitness consequences as a result of genetic stress (such

as the loss of genetic variation due to inbreeding) although with contradictory results (e.g., Møller and Höglund 1991; Gomendio et al. 2000; Bjorksten et al. 2000). Although FA of morphological traits in relation to DI may be both trait- and species-specific (Leung and Forbes 1996; Kark et al. 2004), further studies are needed to examine the effects of FA on genetic stress due to population bottlenecks, to determine if it is useful as a general tool in species conservation.

There have been few studies that have directly examined FA in free-living populations that have passed through bottlenecks of known size, and then related changes in the post-bottlenecked populations back to their source populations. Hoelzel et al. (2002) found a loss of genetic variation along with higher levels of FA in a post-bottlenecked population of northern elephant seals (*Mirounga angustirostris*). However, no studies have examined changes in FA using several species that have passed through various bottleneck sizes, in order to determine how levels of FA change in relation to the severity of a bottleneck.

In the late 1800's numerous avian species were introduced to New Zealand (NZ; Long 1981). Most of these species were introduced from the United Kingdom (UK) and records for the numbers of birds released, along with their release dates, are fairly accurate due to the extensive documentation by the acclimatization societies responsible for their importation (Thomson 1922). This has created an ideal study system in which comparisons with established populations in NZ can be made with the source populations in their native habitat of the UK (Briskie 2006).

In this study, I compared the levels of FA in a variety of external morphological traits of several introduced species to NZ that have passed through population bottlenecks of varying size. Examination of several traits simultaneously allows for a more reliable assessment of FA because DI may be expressed at differing

levels in different morphological traits during an individual's lifetime (Berggren 2005). External traits were used since they are easily measured and the birds can be released without any harm, making them the most useful traits for use in the conservation of endangered birds. I tested the hypothesis that the levels of FA in relation to bottleneck size will differ, such that (1) within-species FA in traits will be greater in introduced (bottlenecked) populations than their source populations, and (2) the level of FA in traits should decrease as the size of the bottleneck increases. Findings of this study may provide insight into the value of using FA as an indicator of DI, and the size of bottleneck at which DI is minimized, both of which could be used to predict possible fitness outcomes of translocation and re-introduction programs of endangered species.

Materials and Methods

Study Areas

FA data were taken from a total of 1146 individuals from ten species of passerines (Table 1.1). Birds were sampled at field sites in the introduced NZ range (Kaikoura, Ward, Blenheim), and in the source range in the UK (Rye Bay Ringing Observatory) from 2005-2006. The only exception was data on Yellowhammers and Cirl Buntings for which I could only obtain NZ samples. The Kaikoura, Ward, and Blenheim study sites are situated along the eastern coast of the South Island in New Zealand (42°23' S 173°37', 41°48' S 174°06' E, 41°28' S 173° 57' E, respectively). They are predominantly agricultural areas, with patches of both introduced and native forest, providing suitable breeding and feeding habitats for most of the study species.

Rye Bay Ringing Observatory is located in East Sussex, on the south east coast of the UK (50°54' N 0°41' E). This site is a wetland reserve that provides a stopover site for many migrating European warbler species, such as the Sedge Warbler (*Acrocephalus schoenobaenus*), and Reed Warbler (*Acrocephalus scirpaceus*). In addition, its forested surroundings, consisting mainly of native deciduous trees, and farmlands provides plenty of breeding and feeding habitats for the study species, allowing sufficient numbers to be captured at that location. Together these locations proved ideal for examining differences in FA, providing suitable numbers to be examined during the course of this study.

Morphometrics and measurement error

Birds were captured from the end of their breeding season through to the end of autumn in each country (February to mid-May in NZ; late August to early November in the UK) using mist nests with a mesh size of 30 mm and ground traps, according to the British Trust of Ornithology, and New Zealand standard protocols. Each individual underwent three repeated measurements of the bill (length from the front of the nares to the tip of the bill), tarsus (from the intertarsal joint to the distal end of the tarsometatarsus), unflattened wing chord (from the radial carpal joint to the tip of the longest primary feather of the closed wing), and the ninth primary feather (hereafter P9; from the base of the feather at the skin to its tip; Fig 1.1). The bill and tarsus lengths were measured using digital calipers (Mitutoyo, Mitutoyo Corporation, Japan) to the nearest 0.01 mm, and the wing chord and P9 were measured using a stopped rule to the nearest 0.5 mm. All measurements were taken by only one person (C.A. Debruyne) to eliminate observer differences or measurement biases. Wing chord and P9 data were only used from individuals which had already completed their

post-juvenal or pre-basic moult to avoid the confounding effect of incomplete feather growth. Age and sex of each individual were determined by plumage characteristics (Svenson 1992). Lastly, individuals of each species were divided into two study groups according to location (either NZ or UK) in order to compare FA between introduced and source populations in NZ and UK, respectively.

Measurement error (ME), the removal of outliers, and testing for the presence of Directional Asymmetry (DA) and antisymmetry were calculated according to Palmer (1994) and Palmer and Strobeck (2003). As suggested, a series of scatterplots of trait measurements followed by Grubb's Tests (Sokal and Rohlf 1995) were conducted to remove any anomalous individuals. To determine if ME was negligible compared to FA, two-way mixed model ANOVAs were conducted for every trait and group separately using the measurements of each side (as a fixed factor), and individuals (as a random factor). In addition, DA and antisymmetry were tested by calculating the skew and kurtosis of the frequency distributions of the between-sides measurements along with three-way ANOVAs (using location, age, and sex, as fixed factors) for every trait examined.

FA indices

FA can be calculated in a variety of ways and I used 4 different estimates of FA: FA1 (the mean absolute value of the mean right minus left measurements), FA4a (the magnitude of FA including ME), FA10a (the magnitude of FA as a proportion of the trait mean after removing ME), and FA8a (the size-scaled estimate of between-sides variance). All measures of FA were calculated according to Palmer and Strobeck (2003). FA4a and FA10a were calculated to verify the robustness of FA1 values and were not used in any of the statistical analyses. Levels of FA between

traits differed significantly ($p < 0.05$ for all traits) during the calculation procedure of the various FA indices therefore following analyses were conducted on a trait-by-trait basis. Subsequent analyses of covariances (ANCOVAs) were conducted (using FA1) to examine within-species FA differences between locations for each trait, considering the effect of age and sex (Palmer and Strobeck 2004).

To examine the relationship between bottleneck severity and change in FA between both locations, least squares linear regressions were performed (on a trait-by-trait basis) using the difference of FA8a (between UK and NZ species) as the dependant variable, and bottleneck size as the independent variable, on all age and sex groups, respectively. FA8a is an absolute relative asymmetry [ln(right - left)] that allows for between-species comparisons of FA. Furthermore, least squares linear regressions were also performed (on a trait-by-trait basis) to examine the relationship between bottleneck severity and FA in species within New Zealand using FA8a as the dependant variable, and bottleneck size as the independent variable, on all age and sex groups, respectively. As some of the species belonged to the same genus (i.e., *Carduelis*: Goldfinch, Greenfinch, and Redpoll; *Emberiza*: Yellowhammer and Cirl Bunting; *Turdus*: Blackbird and Song Thrush), within NZ linear regressions were then reanalyzed using the comparative analysis by independent contrasts (CAIC) software to test for phylogenetic artefacts (Purvis and Rambaut 1994). Bottleneck sizes for NZ populations were provided from acclimatization societies (Lever 1987; Table 1.1). All tests were performed using SPSS (Version 11.04, SPSS Inc. 2005), with a significance level of $p < 0.05$, however, significance of the multiple ANCOVAs were determined using a Bernoulli process which calculates the probability of only one test being statistically significant due to chance alone (Moran 2003).

Results

FA differences between source and bottlenecked populations

A total of 1126 individuals of nine species met all criteria for calculating FA (Palmer and Strobeck 2001; see Appendix A for within-species ME and FA scores). Cirl Buntings were omitted from the analyses due to the presence of DA and ME being greater than FA (mixed-model ANOVAs: $p > 0.05$ for all traits) and a further four individual were omitted as outliers. The ANCOVAs indicated that there was a significant difference in FA between the NZ and UK populations for only one species and one trait examined: FA of the tarsus length of the Redpoll was significantly higher in the UK population compared to NZ ($F_{1,81} = 8.45$, $p = 0.005$, $n = 43$ birds measured in each population; Fig. 1.2), a result that is opposite to what was expected. Using the Bernoulli process, the probability that this was due to chance alone was 0.33. No other species differed significantly between NZ and the UK in any FA score, although FA of the bill length in Greenfinches and of the wing chord in House Sparrows tended towards significance ($p < 0.1$; Table 1.2) and were both higher in the UK populations.

FA in relation to bottleneck size

Least squares linear regression of the difference in FA8a between NZ and UK populations indicated that there was a significant relationship between FA and bottleneck severity of the bill length in hatch year (HY) males, although it was in the opposite direction than predicted ($p = 0.044$, $r^2_{adj} = 0.72$, $n = 5$). In other words, FA

of bill length in NZ populations decreased, in comparison to their UK counterparts, as bottleneck size increased (Fig. 1.3). This comparison suggests lower levels of FA in NZ species that passed through the most severe bottlenecks compared to the UK source populations.

Although there was some evidence of widespread changes in FA between the source populations in the UK and their introduced populations in NZ (albeit in the opposite direction to that predicted), I next determined whether FA varied among NZ populations only in relation to bottleneck size as it is possible that FA in the source populations is also elevated due to recent environmental effects and this may mask patterns present in the NZ populations. As predicted, least squares linear regressions of FA8a within New Zealand populations revealed significant negative relationships between FA and bottleneck severity in the wing chord and P9 length of HY birds. FA of the wing chord in HYs decreased as bottleneck size increased when sexes were pooled ($p = 0.005$, $r^2_{adj} = 0.38$, $n = 9$; Fig. 1.4). This relationship held when controlled for phylogeny ($p = 0.03$, $n = 8$). Similarly, FA of P9 in HY females decreased as bottleneck size increased ($p = 0.049$, $r^2_{adj} = 0.70$, $n = 6$; Fig. 1.5) and this remained significant when controlled for phylogeny ($p = 0.037$, $n = 5$). The rest of the traits examined were non-significant (p values ranged from 0.07 to 0.99). None of these non-significant relationships changed when controlled for phylogeny (p values ranged from 0.34 to 0.99).

Discussion

Hoelzel et al. (2002) noted that there have been few studies of the changes in FA between pre- and post-bottleneck populations. Moreover, FA in avian species that

have passed through differing bottlenecks has been poorly studied; in particular, no previous studies have compared differences in FA in a variety of morphological traits across several species of passerines. In my study I have compared for the first time a variety of within-species traits in FA, and FA in relation to bottleneck severity between introduced species in NZ to their source populations in the UK. I found that levels of FA varied among populations of introduced species in NZ, with those species passing through the most severe bottlenecks exhibiting higher levels of FA than species passing through larger bottlenecks. This is the pattern expected if passing through a severe bottleneck increases inbreeding depression and this in turn leads to developmental problems during growth and moult. Surprisingly, I did not find higher levels of FA in NZ populations of introduced birds compared to their UK source populations, and those few differences present were actually opposite to that predicted. This indicated the UK populations of some species exhibited higher levels of FA than populations of the same species in NZ that had passed through a bottleneck during their establishment. Why should UK populations show higher levels of FA than bottlenecked populations of the same species, especially when there was an indication that more severe bottlenecks within NZ have lead to greater levels of FA?

FA in source and bottlenecked populations

The only difference I found between any NZ population and its source was in UK populations of Redpolls. Somewhat unexpectedly, Redpolls in the UK actually exhibited higher FA of the tarsus length than the NZ population. In addition, although only tending towards significance, UK Greenfinches and House Sparrows also had higher levels of FA in bill length and wing chord, respectively. Reasons for these

findings are not known, however, it can be hypothesized that perhaps some UK populations may be experiencing more environmental stress such as habitat loss, predation pressure, and competition. Unlike the NZ populations of these species, Redpolls have shown a decline in the UK (Fuller et al. 2005) and several studies have reported dramatic declines in many of the UK study species which may be due to changes in farming practices (Siriwardena et al 1998; Krebs et al. 1999). As a result, those species that are declining in the UK (i.e., Redpoll, House Sparrow, Blackbird, Song Thrush, Dunnock, and Goldfinch) may not be good candidates to detect differences in FA in relation to population bottlenecks. Furthermore, there have been numerous studies that have demonstrated higher FA in more environmentally stressed populations. Lens et al. (2002) found higher FA of the tarsus length in critically endangered Taita Thrush populations (*Turdus helleri*) living in highly disturbed habitats. Likewise, Bustnes et al. (2002) found increased levels of FA in wing feathers of Glaucous Gulls (*Larus hyperboreus*) exposed to high levels of environmental pollutants obtained through diet.

In contrast to the declining populations in the UK, NZ populations of all species in my study are stable (and even increasing) and this may indicate a high quality of habitat in the introduced ranges. Although the introduction of mammalian predators to NZ has had detrimental effects on native avifauna and on nestling survival (Craig et al. 2000), there is probably less predation pressure on introduced birds after fledging the nest (Briskie, pers. comm.) as a result of fewer aerial predators than in the UK. Moreover, MacLeod et al. (2008) found higher population densities of all of my study species (except for the Blackbird) in NZ and they suggested that it may have been a result of enhanced niche opportunities, exposure to less extreme weather conditions, and release from natural enemy regulation. Consequently, FA,

although present in NZ populations, might be lower due to better environmental conditions than in the native range. Overall, this may indicate that the levels of FA in both countries are likely to be influenced strongly by environmental effects, and that this may mask the underlying effects of bottleneck size, at least in comparisons between the UK and NZ.

FA and bottleneck size in NZ

Removing possible differences in environmental factors between countries, by examining species within NZ only, revealed that FA decreased when bottleneck size increased in HYs for wing chord and in HY females for P9. These findings concur with Hoelzel et al. (2002) and Aldos et al. (1995) who both found that FA increased in inbred populations. Moreover, significance of FA in wing traits in relation to bottleneck size may be a reliable measure of DI since it directly affects individuals by reducing flight performance that, in turn, may decrease survival (Møller and Nielsen 1997; Swaddle 1997; Hambly et al. 2004). In birds, wing feathers are the most common trait in which asymmetry has been noted (Hambly et al. 2004) and could reflect both genetic and environmental stresses on an individual since random perturbations could affect feathers at both the embryonic stage of development and during feather growth (Stige et al. 2005).

Another factor to consider is possible changes in genetic variability that may have occurred between NZ and UK populations. Previous genetic analyses on introduced species in NZ have shown little or no evidence of changes in average expected heterozygosity (e.g., Ross 1983; Parkin and Cole 1985; Baker and Moeed 1987; Baker 1992; Merila et al. 1996), however improvements on genetic techniques have since been made to increase their accuracy. Clearly, there is a need for further

genetic analyses to quantify the relationship between FA and inbreeding – and to determine if the remaining species of introduced birds to NZ populations display signs of genetic drift or decreased genetic variability.

FA as an indicator of DI

Choosing the proper traits for FA studies as an indicator of DI may also be quite an important consideration. FA in bill and tarsal lengths may not be good indicators of DI due to inbreeding since they may not directly influence reproductive success and survival (e.g., Dufour and Weatherhead 1999). Swaddle (1997) found that an increase in wing asymmetry decreased flight performance in European Starlings (*Sturnus vulgaris*) and he stressed the importance of considering biomechanical performance when studying DI. Aparicio and Bonal (2002) also suggested that traits with fewer structural components might make them more susceptible to FA since less energy is allocated for their development. Although unknown for my study species, future analyses may demonstrate a similar negative relationship between FA and the energetic costs of trait development.

Age and sex differences may also affect FA levels in a population. I found that FA in relation to bottleneck size was only observed in HY individuals. Similarly, Herrando and Brotons (2001) found higher levels of FA in juvenile Sardinian Warblers (*Sylvia melanocephala*) inhabiting lower quality habitats but not in adult populations. Lens et al. (2002) and Møller (1997) proposed that individuals exhibiting high levels of FA might have lower probabilities of survival; therefore high FA levels in HYs are not observed in the AHY population simply because young birds with high FA do not survive to become adults. Karvonen et al. (2003) also found FA differences among age and sex groups of Greenfinches across Europe. He

suggested that males may be more prone to developmental perturbations than females, resulting in higher FA levels than females due to greater energetic requirements needed for growth and higher physiological stressors during development. Although I found some differences in FA between the sexes in relation to bottleneck severity, further examination of the relationship between FA and possible energetic and physiological constraints in my study species would be required to explain my findings. Consequently, all of these factors may be important to consider when evaluating DI of a population in relation to FA and inbreeding.

Implications for conservation

Although I could not demonstrate that observed levels of FA in introduced birds are directly the result of the size of the bottleneck they had passed through upon introduction to NZ, my findings indicated that FA did vary among NZ populations in relation to bottleneck, which may be a subtle indicator of the negative consequences of population bottlenecks. My findings suggest that in order to avoid high levels of FA, large numbers of individuals (1000+) must be used for introductions. In support of this, Briskie and Mackintosh (2004) compared hatching failure in several NZ introduced avian populations to their source populations in the UK and found hatching problems arose in populations founded by fewer than 600 individuals, which demonstrate negative effects of bottlenecks and that they may persist over many generations. In addition, Allen (2008, unpubl. data) found the more severely bottlenecked NZ populations of introduced birds experienced higher levels of ectoparasitism than their UK counterparts, and exhibited increased investment in immune function, (potentially a reflection of increased immune challenges in the bottlenecked populations). Thus patterns of FA observed in NZ populations could be

more evidence in support of the negative consequences of bottlenecks. Moreover, the relative ease of use of this non-invasive method of measuring the negative fitness effects of a bottleneck would make it a practical tool in species conservation, and if applied with other indicators of fitness, such as hatching failure or immunocompetence, FA measures of wing traits may be a useful predictor of DI in a potentially inbred population.

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Figures

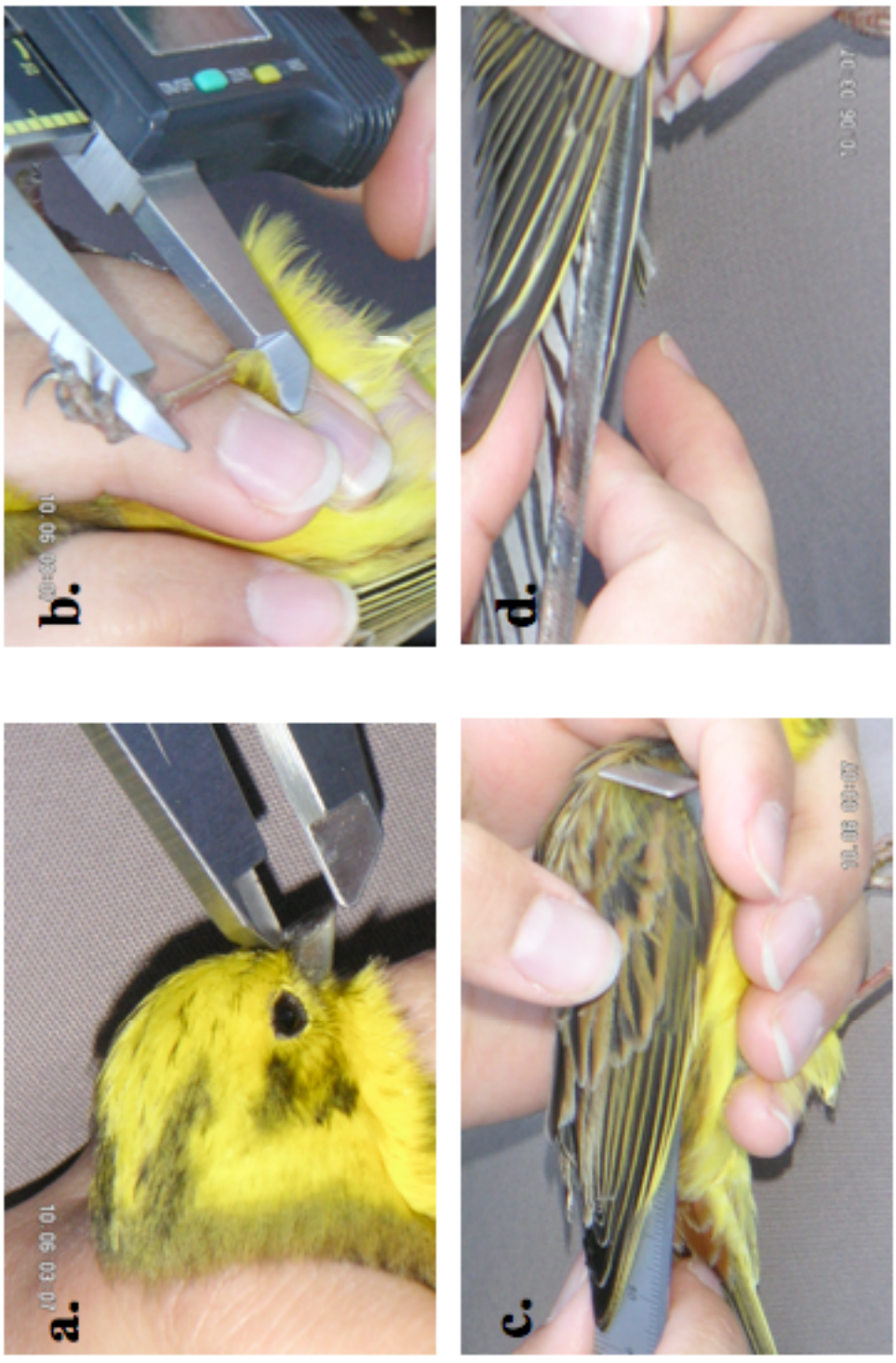

Figure 1.1. Measurements of the a. bill, b. tarsus, c. wing chord, and d. ninth primary (P9) of a Yellowhammer.

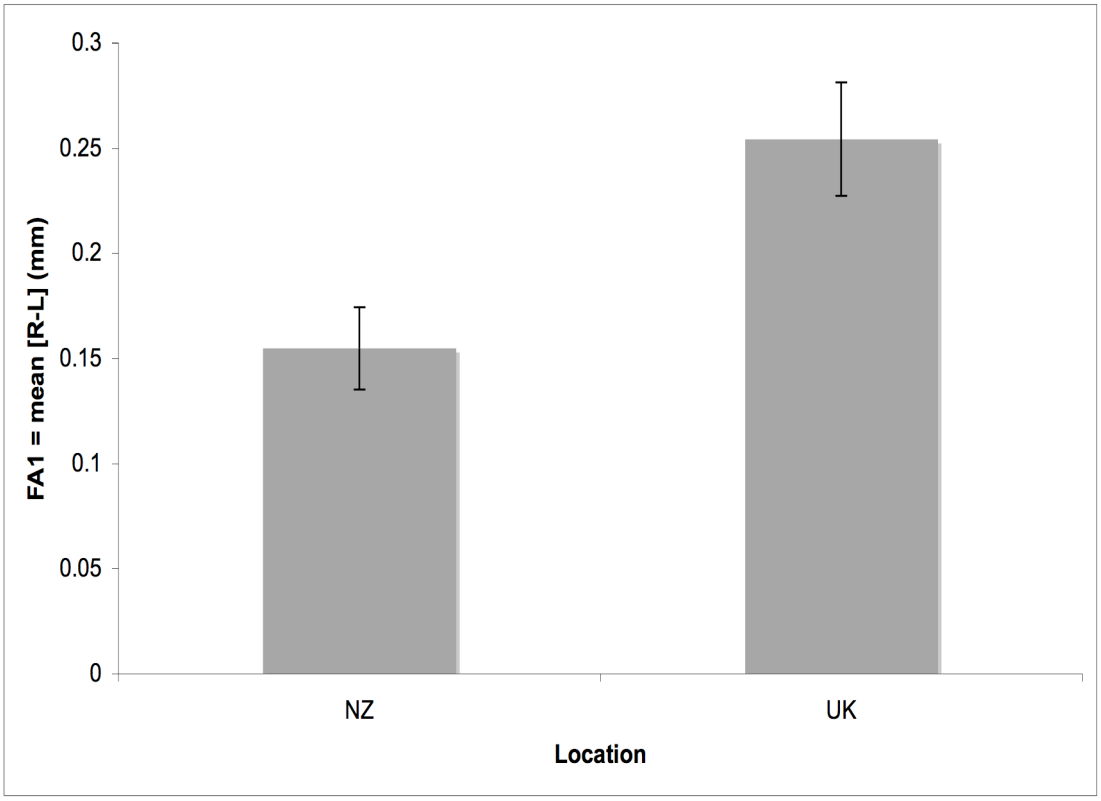

Figure 1.2. Mean FA1 (± S.E.) of the tarsus length of NZ and UK Redpolls.

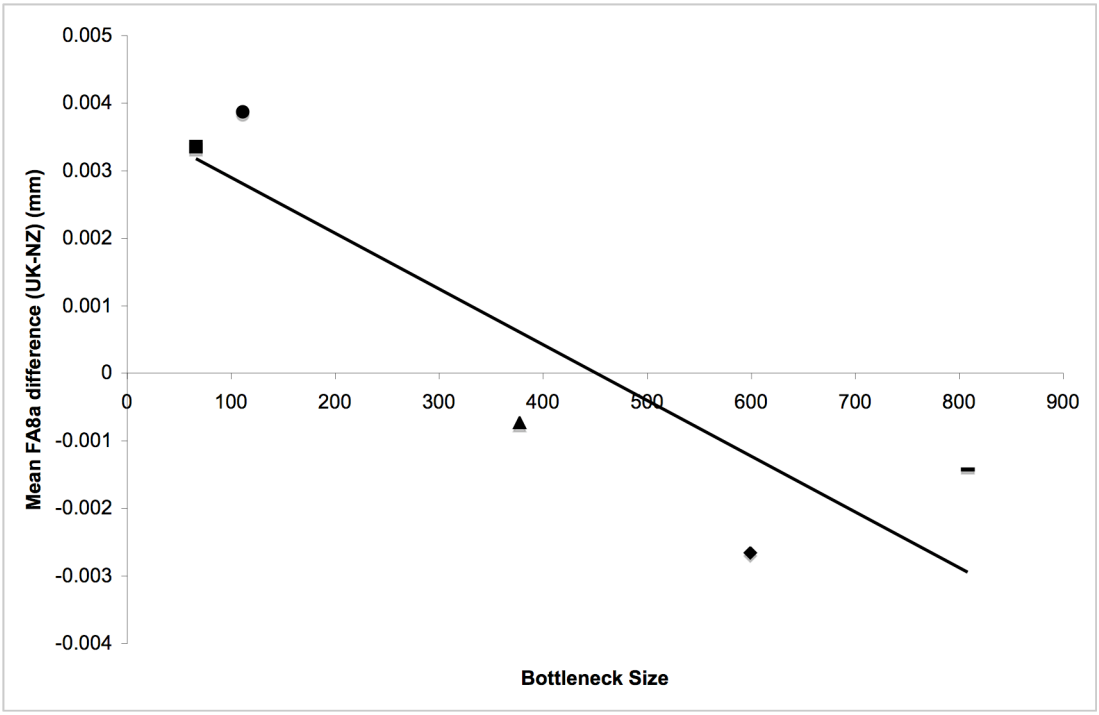

Figure 1.3. Mean FA8a difference of bill length in HY males (■) Greenfinches, (●) House Sparrows, (▲) Chaffinches, (◆) Redpolls, and (-) Blackbirds between NZ and UK populations at varying bottlenecks sizes ($p = 0.044$, $r^2_{adj} = 0.72$, $n = 5$).

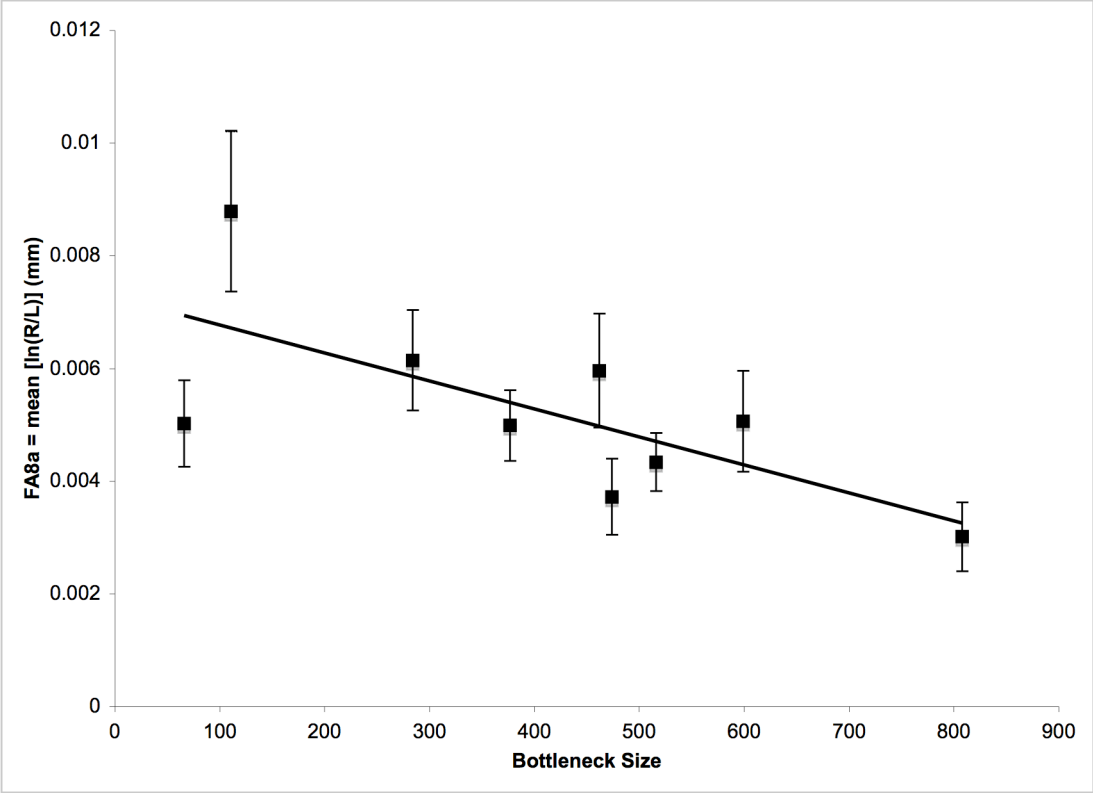

Figure 1.4. Mean FA8a (± S.E.) of the wing chord in NZ HYs from 9 species that have passed through varying bottlenecks.

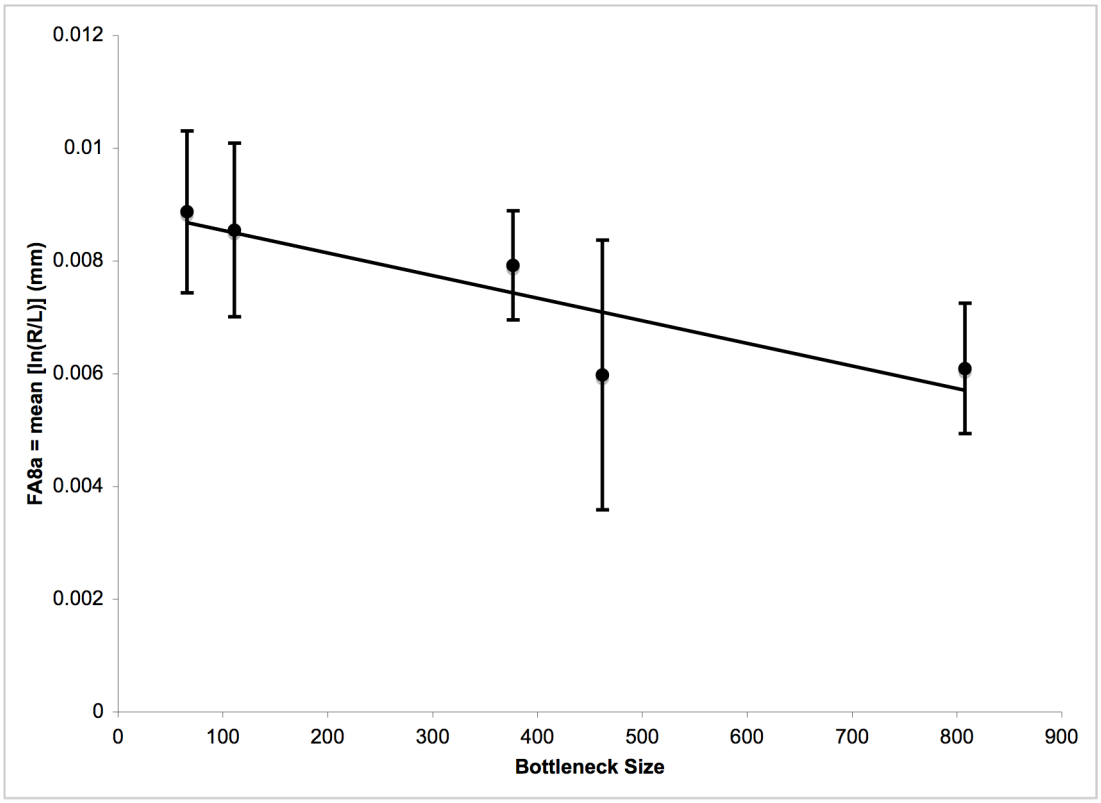

Figure 1.5. Mean FA8a (± S.E.) of P9 in NZ HY females from 5 species that have passed through varying bottlenecks.

Tables

Table 1.1. Number of individuals measured for each species from their source populations in the United Kingdom (UK) and their introduced ranges in New Zealand (NZ). The size of bottleneck (number of individuals introduced) for each species in NZ is also given.

Species	***n***		**Bottleneck Size**
	UK	**NZ**	
Cirl Bunting *Emberiza cirlus*	N/A	16	11
Greenfinch *Carduelis chloris*	72	68	66
House Sparrow *Passer domesticus*	27	125	111
Dunnock *Prunella modularis*	118	54	284
Chaffinch *Fringilla coelebs*	44	108	377
Yellowhammer *Emberiza citrinella*	N/A	41	462
Song Thrush *Turdus philomelos*	57	26	474
Goldfinch *Carduelis carduelis*	88	69	516
Lesser Redpoll *Carduelis flammea*	50	58	599
Blackbird *Turdus merula*	75	50	808

Table 1.2. ANCOVAs (using age and sex as covariates) evaluating the differences in FA1 between NZ and UK populations for the bill length, tarsus length, wing chord, and P9.

Species	**Trait**	**F**	**df**	**P Value**
Blackbird	Bill length	0.660	1,96	0.418
Chaffinch		1.877	1,134	0.173
Dunnock		2.184	1,146	0.142
Goldfinch		0.994	1,122	0.321
Greenfinch		3.462	1,99	0.066*
House Sparrow		0.212	1,123	0.646
Redpoll		0.170	1,86	0.681
Song Thrush		0.267	1,71	0.607
Blackbird	Tarsus length	0.575	1,98	0.450
Chaffinch		0.163	1,130	0.687
Dunnock		0.148	1,145	0.701
Goldfinch		0.034	1,128	0.853
Greenfinch		0.338	1,99	0.535
House Sparrow		0.302	1,125	0.584
Redpoll		8.449	1,81	0.005**
Song Thrush		0.122	1,70	0.728
Blackbird	Wing chord	1.966	1,105	0.164
Chaffinch		0.247	1,137	0.620
Dunnock		0.722	1,148	0.397
Goldfinch		1.798	1,136	0.192
Greenfinch		0.378	1,115	0.540
House Sparrow		3.099	1,134	0.081*
Redpoll		0.912	1,85	0.342
Song Thrush		1.543	1,71	0.218
Blackbird	P9	0.128	1,92	0.721
Chaffinch		0.146	1,124	0.703
Dunnock		1.014	1,144	0.316
Goldfinch		0.775	1,115	0.380
Greenfinch		2.473	1,104	0.119
House Sparrow		1.260	1,82	0.265
Redpoll		0.429	1,74	0.514
Song Thrush		0.470	1,66	0.495

* Significant at $p < 0.1$

** Significant at $p < 0.05$

CHAPTER 2: Fluctuating asymmetry in the skeletal structure of introduced birds in New Zealand

Abstract

The introduction of several avian species to New Zealand (NZ) from the United Kingdom (UK) over one hundred years ago has unintentionally created an ideal study system to examine potential changes in developmental stability due bottleneck effects. In this study I examined fluctuating asymmetry (FA; a common index used to evaluate developmental instability) in several osteological characters of seven avian species introduced to NZ in relation to the bottleneck size they passed through during their establishment. I hypothesized that levels of FA in relation to bottleneck size will differ, such that within-species FA in traits will be greater in introduced (bottlenecked) populations than their source populations, and the level of FA in traits should decrease as the size of the bottleneck increases. Within-species comparisons revealed differences in FA for three species in two characters examined. The FA of the tarsometatarsus length was higher in UK source populations of Greenfinches (*Carduelis chloris*) and House Sparrows (*Passer domesticus*) compared to their NZ counterparts, a pattern opposite to that predicted. Only the FA of the ulna was higher in introduced populations of NZ Dunnocks (*Prunella modularis*). There was no significant relationship between the difference in FA of source and introduced populations in relation to bottleneck size, or when I considered only the populations of introduced species within NZ. Reasons for a lack of a pattern of FA in osteological characters with bottleneck size is unknown, however a combination of possible environmental and genetic stressors may have resulted in species- and character-specific FA relationships. At present, it does not appear that measuring the FA of skeletal characteristics in birds is a useful general tool to measure the potential negative effects of severe population bottlenecks.

Introduction

Many ecosystems around the world are being exploited to meet the needs of humans, and in the process exposing many animal populations to a variety of added, and often detrimental, stresses. Habitat loss or habitat fragmentation due to agriculture and urbanization can divide and isolate larger populations and the stresses this causes can lead a decline in overall numbers which is of great concern in the conservation management of endangered populations. This reduction in the number of individuals from its original population size – termed a population bottleneck – is a common occurrence in today's environment (Frankham et al. 2002), and is predicted to become even more frequent as human population expands.

Severe population bottlenecks can have adverse effects on a population by lowering heterozygosity, increasing expression of deleterious genes in future generations, and increasing the likelihood of inbreeding (Nei et al. 1974; Frankham et al. 2002). Moreover, fitness levels of bottlenecked populations may be compromised and individuals within a bottlenecked population may show signs of developmental instability (DI), which is defined as the inability to endure random genetic or environmental perturbations during development (Parsons 1992; Palmer and Strobeck 1992; Lens et al. 2002). This in turn could decrease the probability of maintaining (or establishing) a stable population.

Introduced populations, whether founded intentionally for species conservation, or through self-introduction, often pass through a bottleneck. New Zealand (NZ) has a very large number of introduced organisms, including over thirty species of exotic birds (Thomson 1922; Long 1981). This is the result of various acclimatization programs in the late 1800's which lead to the introduction of

numerous avian species, primarily from the United Kingdom (UK; Long 1981). The organized and institutional nature of the acclimatization societies meant that fairly accurate records were made for the numbers of birds released, along with their release dates (Thomson 1922). As a result, this has created an ideal study system for comparing established populations of a variety of introduced species in NZ with their source populations in their native habitat of the UK (Briskie 2006).

One potential consequence of a population bottleneck is an increase in the levels of developmental problems encountered by individuals in the post-bottlenecked population due to inbreeding depression (e.g., Keller and Waller 2002). An increase in fluctuating asymmetry (FA) might be expected after a population has passed through a severe bottleneck. FA is the random deviations from symmetry between a bilaterally symmetrical trait (Van Valen 1962; Palmer and Strobeck 1986) and it has become a commonly used measure of DI in various species (e.g., Rintamäki et al. 1997; Polak 1997). However, only a few studies have directly examined FA in populations that have passed through bottlenecks of known size, and have related any differences in the levels of FA to their source populations. For example, Hoelzel et al. (2002) found higher levels of FA in a variety of skeletal measures of the skull in a post-bottleneck population of northern elephant seals (*Mirounga angustirostris*). This species had passed through a severe bottleneck due to over-hunting in the 19th century, and although the population had now recovered, individuals in the post-bottlenecked population expressed developmental problems as a result.

Measurements of FA have been used in a variety of contexts apart from conservation management but have had mixed results (e.g., Møller 1992; Bergström and Reimchen 2000; Bjorksten et al. 2000) and there is a need for further examination of FA of morphological traits as a potential indicator DI. Part of the reason for a lack

of consistency in studies using FA as a measurement tool is that FA may be both trait- and species-specific (Leung and Forbes 1996; Kark et al. 2004). Moreover, certain traits may have higher levels of FA due to the complexity of their structural composition (Aparicio and Bonal 2002), and increased energetic investment in life history traits that may be essential for survival.

In this study, I examined levels of FA in a variety of skeletal characters of introduced birds in NZ in relation to their population bottleneck size. The species in my study passed through population bottlenecks during their establishment that varied in size from 11 to 808 individuals and I used this fact to assess if bones can be used as a reliable indicator of DI in relation to bottleneck effects. The simultaneous examination of several skeletal characters allowed for a more reliable assessment of FA because DI may be expressed at differing levels in different morphological traits during an individual's lifetime (Berggren 2005). Furthermore, bone growth is likely influenced during embryonic and nestling development (Maul and Farris 2005), and therefore measures of FA in skeletal traits may be a useful index of long-term stresses an individual may be experiencing. For most endangered species that have passed through severe bottlenecks, the source (or pre-bottlenecked) population is no longer available for comparison with present day populations, but I was able to use museum skeletal collections from the source populations to examine the effects of population bottlenecks on developmental stability. My objective was thus to provide a better understanding of FA as an indicator of DI, which could be used as a practical tool in species conservation and the management of bottlenecks.

Materials and Methods

FA data ware taken from measurements of a total of 186 skeletons from seven species of passerines (Table 2.1). Measurements from birds in the source populations were obtained from specimens housed at the Natural History Museum in Tring, UK (90 skeletons). Measurements from birds in the introduced populations in NZ were collected and prepared by myself from carcasses I collected or from window-strikes donated to the University of Canterbury (96 skeletons). The only exception was the Cirl Bunting, in which only NZ samples were available. All NZ specimens were collected from 2000-2006. UK specimens had a mean collection year of 1977. The NZ carcasses were processed into skeletons using a dermestid beetle (*Dermestidae spp.*) colony. Each carcass was skinned, gutted, and then placed into a sealed aquarium containing the beetles for approximately three days to allow sufficient time for removal of all tissues. Skeletons were then removed from the tank, washed with a mild detergent, bleached in 0.01 mol solution of hydrogen peroxide for twenty-four hours, then washed again, and set to dry for five days before handling. The cleaned and disarticulated skeletons were then stored in separate labelled containers until measured.

Morphometrics and measurement error

For each skeleton I measured the length of the following bones: humerus, radius, ulna, femur, tibia, tarsometarsus, sternum, and illium (Fig. 2.1). Each measurement was repeated 3 times, and bones from both the left and right side were measured. Individual bones were measured using digital calipers (Mitutoyo,

Mitutoyo Corporation, Japan) to the nearest 0.01 mm. All measurements were taken by only one person (C.A. Debruyne) to eliminate observer differences or measurement biases. Lastly, each species was divided into two groups according to collection location (either NZ or UK) in order to compare FA between introduced and source populations, respectively.

Measurement error (ME), the removal of outliers, and testing for the presence of Directional Asymmetry (DA) and antisymmetry were calculated according to Palmer (1994) and Palmer and Strobeck (2003). As suggested by these authors, a series of scatterplots of trait measurements followed by Grubb's Tests (Sokal and Rohlf 1995) were conducted to remove any outliers and anomalous individuals. To determine if ME was negligible compared to FA, two-way mixed model ANOVAs were conducted for every trait and group separately using the measurements of each side (as a fixed factor), and individuals (as a random factor). In addition, DA and antisymmetry were tested by calculating the skew and kurtosis of the frequency distributions of the between-sides measurements along with t-tests (comparing the mean [right minus left] to zero) for every trait examined.

FA indices

FA can be calculated in a variety of ways and I used 4 different estimates of FA: FA1 (the mean absolute value of the mean right minus left measurements), FA4a (the magnitude of FA including ME), FA10a (the magnitude of FA as a proportion of the trait mean after removing ME), and FA8a (the size-scaled estimate of between-sides variance). All measures of FA were calculated according to Palmer and Strobeck (2003). FA4a and FA10a were calculated to verify the robustness of FA1 values and were not used in any of the statistical analyses. Levels of FA between

traits differed significantly ($p < 0.05$ for all traits) during the calculation procedure of the various FA indices therefore following analyses were conducted on a trait-by-trait basis. Subsequent analyses of covariances (ANCOVAs) were conducted (using FA1) to examine within-species FA differences between locations for each trait, considering the effect of age and sex (Palmer and Strobeck 2003).

To examine the relationship between bottleneck severity and change in FA between source and introduced locations, least squares linear regressions were performed (on a trait-by-trait basis) using the difference of FA8a (excluding Cirl Buntings as there were no UK samples) as the dependant variable, and bottleneck size as the independent variable. FA8a is an absolute relative asymmetry [ln(right - left)] that allows for between-species comparisons of FA. Age and sex groups were pooled due to small sample sizes. Furthermore, least squares linear regressions were also performed (on a trait-by-trait basis) to examine the relationship between bottleneck severity and FA in species within New Zealand (including Cirl Buntings) using FA8a as the dependant variable, and bottleneck size as the independent variable. Within NZ linear regressions underwent comparative analysis by independent contrasts (CAIC) to test for phylogenetic artefacts (Purvis and Rambaut 1994). Bottleneck sizes for New Zealand populations were provided from acclimatization societies (Lever 1987; Table 2.1). All tests were performed using SPSS (Version 11.04, SPSS Inc. 2005), with a significance level of $p < 0.05$, however, significance of the multiple ANCOVAs were determined using a Bernoulli process which calculates the probability of only one test of being statistically significant due to chance alone (Moran 2003).

Results

FA differences between source and bottlenecked populations

A total of 183 individuals met all criteria for calculating FA, however due to the presence of DA in measurements of the ilium, this bone was omitted from further analyses, and 3 individuals were omitted as outliers (Palmer and Strobeck 2003; see Appendix B for within-species ME and FA scores). The ANCOVAs indicated that there were only significant differences in FA1 between NZ and the UK populations for three species in two traits examined. FA of the tarsometatarsus length of Greenfinches ($F_{1,14} = 14.81$; n = 12 skeletons from NZ and 6 skeletons from the UK; $p = 0.002$; Fig. 2.2) and House Sparrows ($F_{1,17} = 4.551$, n = 11 skeletons from NZ and 10 skeletons from the UK; $p = 0.048$; Fig 2.3) were higher in the UK populations compared to NZ, a pattern opposite to that predicted if passing through a bottleneck increases FA. In contrast, FA of the ulna of Dunnocks was higher in the NZ population compared to their UK counterparts ($F_{1,14} = 9.53$, n = 7 skeletons from NZ and 11 skeletons from the UK; $p = 0.008$, Fig. 2.4). Using the Bernoulli process, the probability that these were significant due to chance alone was 0.19.

FA in relation to bottleneck size

Least squares linear regressions of the difference of FA8a between NZ and UK and bottleneck size were not significant ($p > 0.05$ in all cases; Table 2.2). There were also no significant relationships between bottleneck size among the New Zealand populations ($p > 0.05$ in all cases; Table 2.3) for any of the skeletal traits

examined. These results did not change when analyses were controlled for possible phylogenetic effects (all p values > 0.27), and thus I found no evidence that FA of skeletal traits was related to bottleneck size.

Discussion

In this study, I used the comparative method to examine FA of a variety of skeletal traits in introduced species of birds that passed through bottlenecks of differing size. My findings suggest that although some skeletal characters showed differences in FA between source populations in the UK and introduced populations in NZ, these differences were not explained by bottleneck severity. Out of seven species and eight osteological characters examined, only two characters showed significant differences in FA and this was found in only three species. For two species the pattern of FA was actually opposite to that expected, with higher levels of FA in the source populations than in the introduced and post-bottlenecked populations in NZ. Thus, I found little support for the prediction that FA should be consistently higher in NZ populations of introduced birds, and that the degree of FA should increase with the severity of bottleneck size.

FA in source and bottlenecked populations

Understanding FA patterns in morphological traits has the potential to offer insight into possible genetic and environmental factors that play a role in DI, and there have now been a number of studies that have demonstrated higher FA in more environmentally stressed populations. For example, Lens et al. (2002) found higher

levels of FA of the tarsus size of critically endangered Taita Thrush populations (*Turdus helleri*) living in highly disturbed habitats. However, in my study of within-species FA differences between populations before and after a bottleneck, only two skeletal characters in three of the study species were significantly different. It is unclear why I failed to detect a larger and more widespread increase in FA in the NZ populations of each species, although there may be several explanations for the patterns of FA observed.

First, throughout my study I have assumed that the UK populations of the species I studied are under less stress than in NZ populations as a consequence of the latter having passed through bottlenecks, some of which were as few as a dozen individuals. This may not be the case if levels of environmental stress such as habitat loss, predation pressure, and competition are actually higher in the UK than in NZ. For example, House Sparrows have shown a recent severe decline in the UK (Siriwardena et al. 2002; Robinson et al. 2005), and several studies have reported dramatic declines in many of the UK populations of study species examined that may be due to changes in farming practices (Siriwardena et al. 1998; Krebs et al. 1999). Thus it is possible the higher levels of FA found in two UK populations of species in my study may be indicative of greater stress than in introduced populations of the same species.

Second, it is possible that skeletal FA due to bottlenecks may not have strong fitness consequences or that species in different populations may differ in how they trade-off coping with stresses due to bottlenecks. For example, UK and NZ populations might differ in the way they invest and trade-off other life history traits such as immunocompetence, reproductive success, and predator avoidance in order to tolerate existing levels of environmental stress. This might lead to some characters

such as the tarsal bones having higher FA in one population than the other. As a result, environmental stress may still heavily impact levels of FA in avian species but that it can be difficult to find traits that can be reliably compared among different populations and species.

The only species in which I found higher levels of FA was in the ulna of Dunnocks: this species went through a rather severe bottleneck during its establishment (284 individuals) and as predicted the level of FA was higher in NZ compared to their UK counterparts. Wing traits are important for survival since for most birds it is their primary method of locomotion, allowing for prompt escape from predators, access to remote feeding areas, and higher quality habitats through migration and dispersal (Norberg 1979; Pennycuick 1989). Moreover, Swaddle (1997) found that an increase in wing asymmetry decreased flight performance in European Starlings (*Sturnus vulgaris*) and he further stressed the importance of considering biomechanical performance when studying DI. Although the effect of higher FA in the ulnar bones on flight performance is unknown and requires further analysis, increased wing FA has been observed in birds under increased environmental stress (e.g., Bustnes et al. 2002; Brown and Brown 2002). Whether Dunnocks in NZ are under greater stress than in the UK is unknown although I have made the assumption that the bottleneck they passed through during their establishment should have subjected them to more genetically-related stresses than in their native range. Population densities of Dunnocks in NZ are higher than in the UK, which may be a result of enhanced niche opportunities, exposure to less extreme weather conditions, and release from natural enemy regulation (MacLeod et al. 2008), but the higher density could also then exacerbate the stress of a bottleneck through density-dependant effects.

If the higher FA levels in NZ Dunnocks are due to this population experiencing genetic stress, this would be evidence for a negative effect of the population bottleneck they passed through. Negative fitness consequences due to bottleneck severity have already been observed in introduced birds to NZ. For example, Briskie and Mackintosh (2004) compared hatching failure in several NZ introduced avian populations (including Dunnocks) to their source populations in the UK and found hatching problems arose in populations founded by fewer than 600 individuals, which suggests that bottleneck effects may persist over many generations. In addition, I found (Chapter 1) that FA in external wing chord increased as bottleneck severity increased in birds (including Dunnocks) introduced into NZ.

Although I did not find any correlations between FA and bottleneck severity across species, FA in bones in relation to DI due to bottleneck effects may be species specific. It is possible that the Dunnock may be more susceptible to genetic stress than other birds species examined however, there have been no studies examining possible changes in genetic variation between the NZ and UK populations of this species. Previous genetic analyses on introduced species in NZ have shown little or no evidence of changes in average expected heterozygosity (e.g., Ross 1983; Parkin and Cole 1985; Baker and Moeed 1987; Baker 1992; Merila et al. 1996), however improvements on testing has since been made to increase their accuracy thus further analyses are needed to ascertain possible genetic changes in NZ Dunnocks and the other introduced species.

FA in bones as an indicator of DI

Throughout my study, I have used FA of skeletal traits as a measure of DI. This assumption would appear reasonable at first glance given its successful

application in other species (e.g., Hoelzel et al. 2002) but it is possible that measures of FA in the bones I used in my study may not be a good indicator of DI due to bottleneck effects. This might be the case if FA in these bones does not directly influence fitness such as through decreased reproductive success and survival (e.g., Dufour and Weatherhead 1999). Unfortunately, there is little information available on the potential fitness costs of high FA in skeletal traits although the expectation is that high FA would incur higher costs. Indeed, if fitness costs due to FA are high, this might lead to the rapid loss of these individuals from the populations (introduced and source) making FA difficult to detect.

A further problem with using skeletal material for studies of FA is that information about the source, or pre-bottlenecked populations are often only obtained through museum specimens. Swaddle et al. (1994) noted that there might be a collection bias towards more 'aesthetic' samples for museum specimens or that FA may be a result of wear and damage by researchers repeatedly handling the material. Although only the UK population examined came from museums specimens, it is probably unlikely that there is a bias due to appearance since most specimens were collected in the autumn, hence were not in their 'aesthetically pleasing' breeding plumage. Such a collection bias also could not account for higher FA observed in the NZ population of Dunnocks.

In addition, bones may not exhibit true fluctuating asymmetry as Olsen et al. (2000) indicated that vertebrate bones are capable of significant remodelling due a complex regulation system of bone growth by the bone plates. As a result, Palmer and Strobeck (2003) suggested that this may cause deviations from symmetry which could be a sign of phenotypic plasticity – phenotypic changes as a result of changes in environmental conditions – rather than FA, which does not provide information about

DI. However in most cases, bones may be the only source of information available for pre-bottlenecked populations, thus further study is required to examine the relationship between phenotypic plasticity and FA, and possible methods of differentiation between the two.

In summary, I found that FA in bones did not appear to be related to bottleneck size. Nevertheless, higher levels of FA in bones may be an indicator of DI in some species and some traits but reasons for this lack of generality is unknown. Conservation programs should therefore proceed with caution if using FA of skeletal characters in order to assess levels of DI. If FA is used then it is important to choose traits that are of biological relevance to the fitness of the species concerned. Continued examination of the many genetic and environmental aspects of DI will contribute to a better understanding of FA in avian species.

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Figures

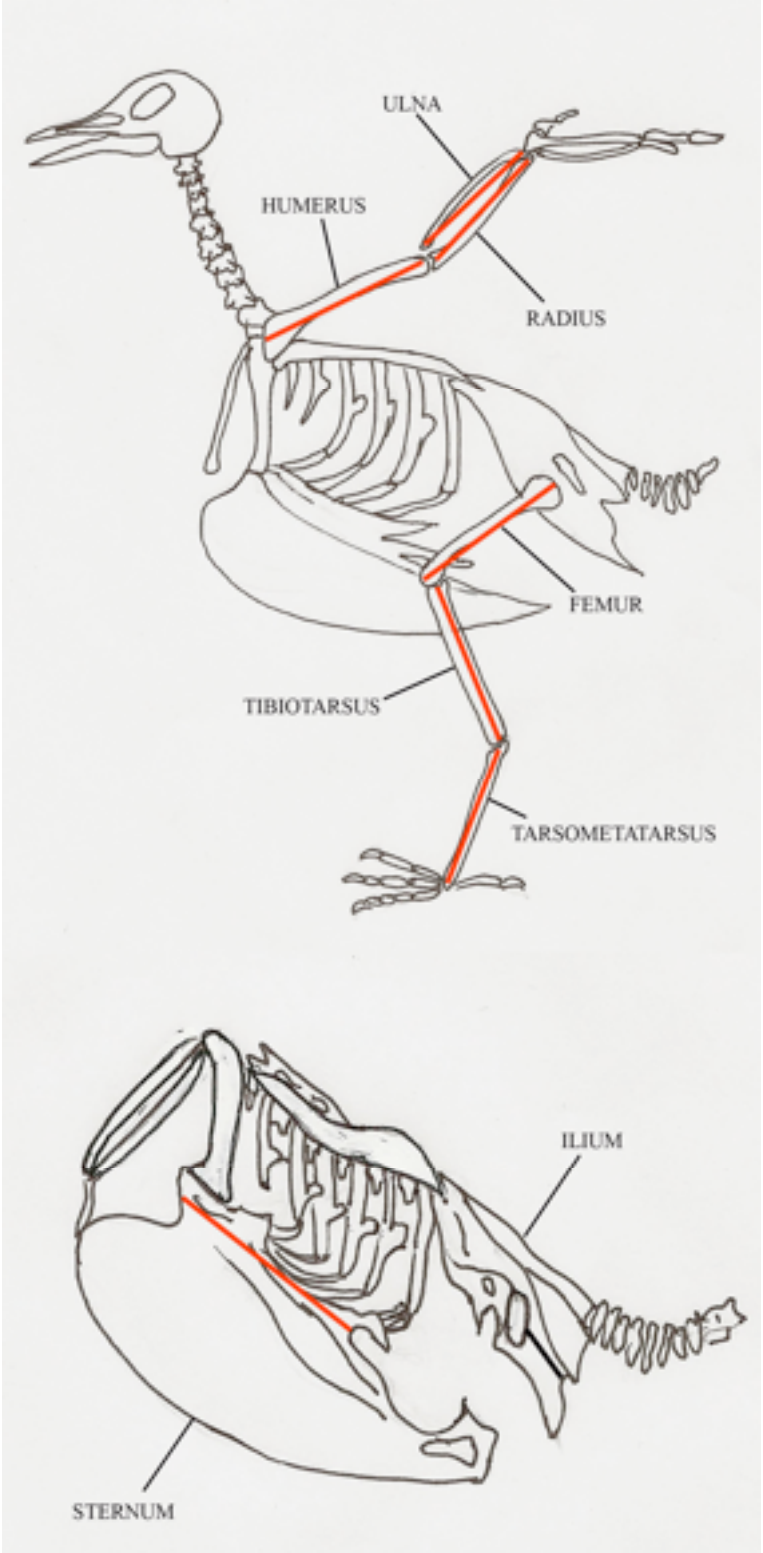

Figure 2.1. Illustration of the eight osteological characters measured on an avian skeleton (dimensions measured are indicated in red). Drawings by Michel Debruyne after D. G. Mackean (2008; top illustration) and Proctor and Lynch (1993; bottom illustration).

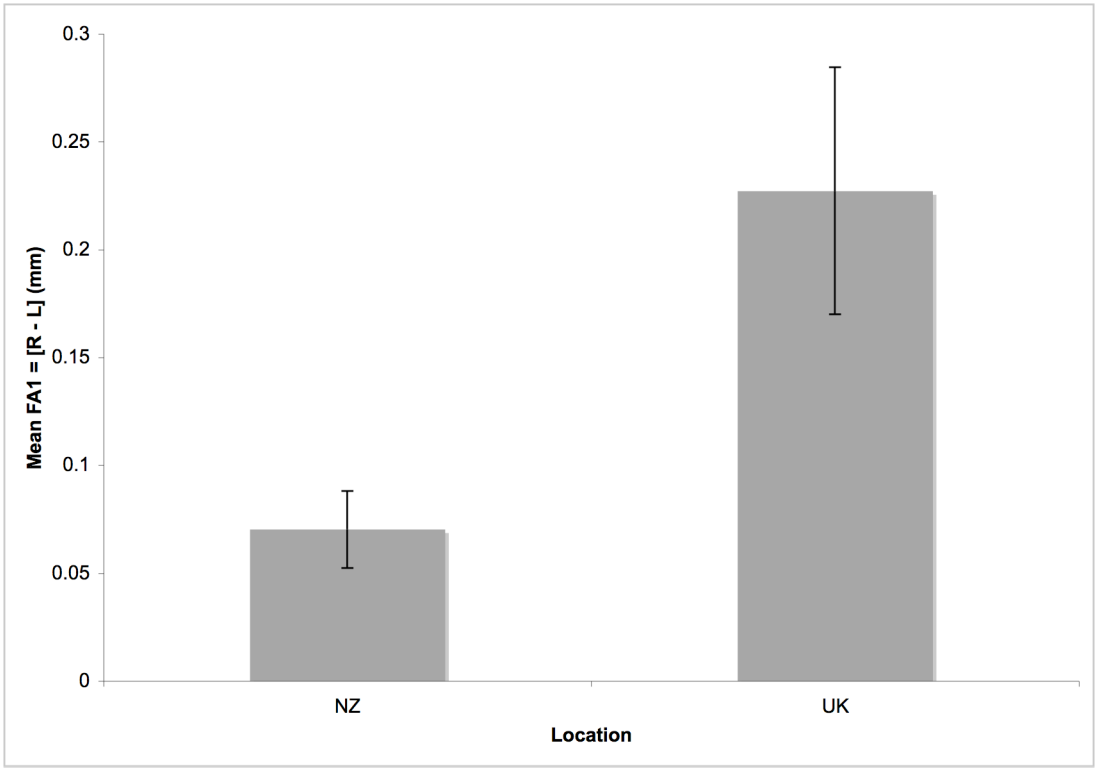

Figure 2.2. Mean FA1 (± S.E.) of the tarsometatarsus length in NZ and UK Greenfinches ($F_{1,14}$ = 14.81; n = 12 NZ, n = 6 UK; p = 0.002).

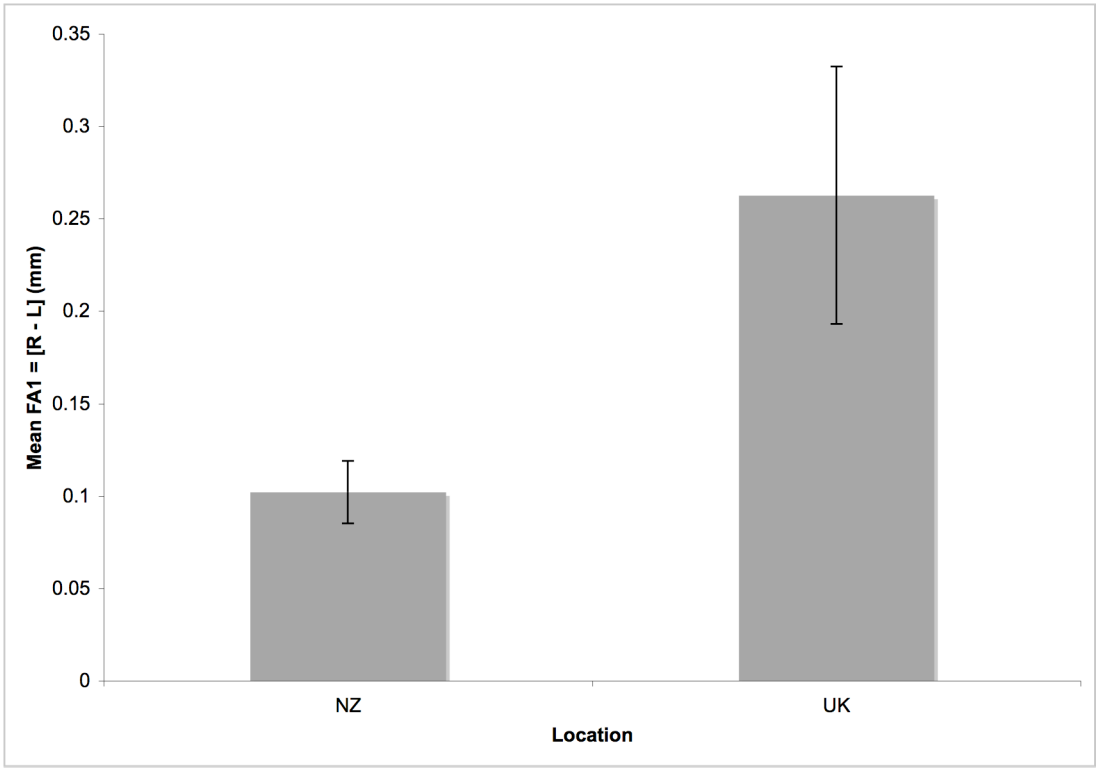

Figure 2.3 Mean FA1 (± S.E.) of the tarsometatarsus length in NZ and UK House Sparrows ($F_{1,17} = 4.551$, n = 11 NZ, n = 10 UK; p = 0.048).

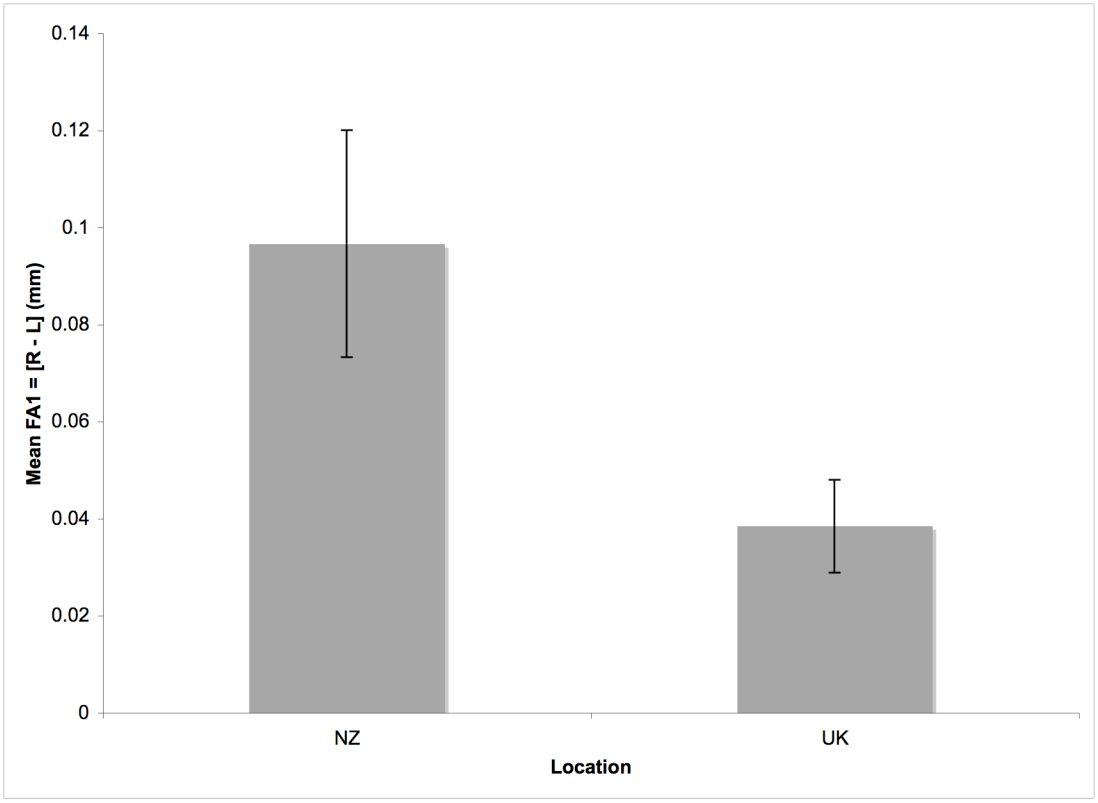

Figure 2.4. Mean FA1 (± S.E.) of the ulna in NZ and UK Dunnocks ($F_{1,14}$ = 9.53, n = 7 NZ, n = 11 UK; p = 0.008).

Tables

Table 2.1. Number of skeletons measured for each species from their source populations in the United Kingdom (UK) and their introduced ranges in New Zealand (NZ). The size of bottleneck (number of individuals introduced) for each species in NZ is also given.

Species	***n***		**Bottleneck Size**
	UK	**NZ**	
Cirl Bunting *Emberiza cirlus*	N/A	11	11
Greenfinch *Carduelis chloris*	13	17	66
House Sparrow *Passer domesticus*	16	15	111
Rook *Corvus frugilegus*	16	13	212
Dunnock *Prunella modularis*	15	9	284
Yellowhammer *Emberiza citrinella*	15	14	462
Blackbird *Turdus merula*	15	17	808

Table 2.2. Relationship between the mean differences in FA8a between introduced and source populations in relation to bottleneck severity. The n, r^2_{adj}, and P value of each regression between bottleneck size and the FA of each of the lengths of seven osteological characters is given.

Character	**n**	**r^2_{adj}**	**P Value**
Femur	6	-0.24	0.87
Humerus	4	-0.37	0.71
Radius	6	-0.24	0.84
Sternum	6	-0.87	0.48
Tarsometatarsus	6	-0.25	0.98
Tibia	5	-0.33	0.90
Ulna	4	-0.39	0.73

Table 2.3. Relationship between the mean FA8a within each population of introduced species in NZ and bottleneck severity. The n, r^2_{adj}, and P value of each regression between FA and bottleneck size is given for each of the lengths of seven osteological characters measured.

Character	**n**	**r^2_{adj}**	**P Value**
Femur	7	-0.18	0.81
Humerus	5	-0.27	0.73
Radius	7	-0.10	0.52
Sternum	7	0.17	0.19
Tarsometatarsus	7	-0.17	0.71
Tibia	6	-0.13	0.55
Ulna	5	-0.30	0.80

CHAPTER 3: Fluctuating asymmetry in the nestlings of Common Mynas and European Starlings introduced to New Zealand

Abstract

The European Starling (*Sturnus vulgaris*) and the Common Myna (*Acridotheres tristis*) are two of the many avian species introduced to NZ in the 19th century. These two species are closely related but passed through different-sized population bottlenecks during their establishment (severe in Mynas, less severe in Starlings). Both species are now common in NZ, allowing for comparisons of bottleneck effects without the confounding effects of phylogeny that are common in other comparative studies. In this study I compared differences in levels of fluctuating asymmetry (FA; random deviations from symmetry between a bilaterally symmetrical trait) in Starling and Myna nestlings in three external morphological traits during nestling development. There were no significant differences in FA between the two species even though it was predicted that Mynas would have greater FA since they passed through a more severe bottleneck. However, FA did change through the nestling period in both species, and some of the traits followed a similar trend as demonstrated in previous studies with other species. FA in all traits also decreased over the nestling period but did not converge by the final day of measurement. Differences in bottleneck severity between Mynas and Starlings did not have an apparent affect on FA in their nestlings, however there may be other environmental stressors that might also influence FA in both species differently, masking potential bottleneck effects.

Introduction

The introduction of foreign flora and fauna, whether for intentional purposes such as species conservation, by accident or through self-introductions, has been a common occurrence in New Zealand (NZ) over the last century (see review in Craig et al. 2000). In the late 1800's numerous avian species were introduced to NZ from various parts of the world including the United Kingdom (UK), North America, and Australia (Long 1981). Records for the numbers of birds released, along with their release dates, are fairly accurate due to the extensive documentation by the acclimatization societies responsible for their importation (Thomson 1922). As a result, this has created an ideal study system for comparison of introduced populations in NZ to their source populations in their native habitats (Briskie 2006).

Two of the successful species – the Starling (*Sturnus vulgaris*) and the Common Myna (*Acridotheres tristis*) – were both released in NZ in the 19th century and are now common. Both belong to the family Sturnidae (Faere and Craig 1999), and have similar life histories (e.g., both are cavity nesting, both are socially monogamous, etc.). Approximately 650 Starlings were brought from the UK to various different regions of the North and South Islands of NZ over a period of twenty years (Lever 1987). They are now abundant throughout NZ, probably as a result of their flexible and adaptive behaviour (Sol and Lefebvre 2000; Clergeau and Quenot 2007). The Myna is native to India, but the Mynas introduced to NZ were sourced from an established introduced population in Australia (Baker and Mooed 1987). Small populations of fewer than 100 individuals were released in the Christchurch, Dunedin, and Nelson regions of the South Island (Thompson 1922; Lever 1987), however due to climatic constraints (the native range of this species is more tropical

than that of the starling), they did not manage to successfully establish themselves there. However, approximately 70 individuals were released in the Wellington and Hawke's Bay area over the period of a few years and since have successfully established throughout the North Island (Lever 1987; Baker and Moeed 1987).

As with all introduced species in NZ, both the Myna and Starling passed through a population bottleneck (a decrease in individuals from its original population size) during their establishment, although the number of founders (and hence severity of bottleneck) differed between the species, with the Myna having passed through bottlenecks both in Australia and New Zealand. Severe population bottlenecks can have a number of detrimental effects on individuals by lowering heterozygosity, increasing expression of deleterious alleles in future generations, and increasing the likelihood of inbreeding (Nei et al. 1974; Frankham et al. 2002). Furthermore, inbreeding depression (a decrease in fitness of a population due to inbreeding; Wright 1977; Shields 1987; Crnokrak and Roff 1999) can lead to an increase in developmental instability (DI), which is the inability to cope with random genetic or environmental perturbations during development (Parsons 1992; Palmer and Strobeck 1992; Lens et al. 2002). Such DI is expected to be greater in individuals in populations that have passed through the most severe bottlenecks. Due to differences in the severity of bottleneck size, Starlings and Mynas are ideal species to examine the potential effects of population bottlenecks on DI, as Myna (having passed through a more severe bottleneck) are expected to show greater levels of DI than Starlings, yet their close phylogenetic relationship limits differences between the species that are inherent in comparative studies with widely-divergent species.

One potential fitness consequence of a severe population bottleneck is an increase in the levels of developmental problems encountered by individuals in the

post-bottlenecked population due to inbreeding depression (e.g., Hoelzel et al. 2002). An increase in fluctuating asymmetry (FA) is one such problem that might be expected to increase after a population has passed through a severe bottleneck. FA is the random deviations from symmetry between a bilaterally symmetrical trait (Van Valen 1962; Palmer and Strobeck 1986) and has become a commonly used measure of DI. FA has been used in numerous studies to assess both environmental and genetic stress, such as pesticide exposure and reproductive success (e.g., Rintamäki et al. 1997; Gomendio et al. 2000; Maul and Farris 2005).

Most studies that have used FA to study stress have generally been limited to a single fixed period, which may not be representative if DI is expressed at differing levels in different morphological traits during different stages an individual's lifetime (Berggren 2005). Aparicio (1998; 2001) suggested that the ontogeny of FA could depend on the growth pattern of the trait; as most traits show determinate growth, this means FA should follow a parabolic function and be at its lowest when the trait reaches its maximum size. For example, Teather (1996) found this pattern of FA in the tarsus length and primary feather length of nestling Tree Swallows (*Tachycineta bicolor*), with FA reaching a peak towards the mid- to later part of the nestling period. Swaddle and Witter (1997) suggested that variations in FA might be related to the functional importance of each trait and should be lower in those traits that are more critical for survival. They also suggested that other mechanisms such as compensational growth (a regulatory growth signal between both sides of the trait) may also affect levels of FA during trait development. As a result, various factors may influence FA and further analyses are warranted to determine if similar patterns are observed in other species.

In this study, I compared differences in the levels of FA in Starlings and Mynas. As these two species have passed through two different-sized population bottlenecks, I tested the hypothesis that the levels of FA will differ between the two species. I predicted that FA in traits of Myna nestlings should be greater than the same traits in Starlings as the former passed through a more severe bottleneck. In addition, I examined if levels of FA were similar among traits, or if some traits were more likely to show higher FA, and whether the levels of FA followed a parabolic pattern during their development. My objective was to increase our understanding of FA in a variety of morphological traits throughout their development and thus whether FA is a useful indicator of DI in endangered species that have passed through severe population bottlenecks.

Materials and Methods

Study Areas

I measured levels of fluctuating asymmetry in Starlings and Mynas nesting at Limestone Downs sheep and cattle farm, and surrounding farms in the Waikato region of New Zealand (NZ) during the summer of 2006. The farms are situated on west coast of the North Island approximately 13 km from the town of Port Waikato (37°29' S, 174°46' E). They are large agricultural areas, with patches of both introduced and native forest, providing suitable breeding and feeding habitats for both study species. Together these locations proved ideal for examining differences in nestling FA during the course of this study.

Field Procedures, morphometrics, and measurement error

A total of 180 nest boxes (100 Myna, 80 Starling) were erected throughout the various field sites during the winters of 2006 and 2007. The dimension of each box was approximately 20 cm x 20 cm x 40 cm and was made from weather-treated wood with a removable lid for easy access inside. Boxes had either a 50 mm (for Starlings) or 65 mm (for Mynas) circular opening at top of the box front to allow access for the birds (although both species were found to nest in boxes of either type despite the entrance size). Nest boxes were attached on trees or in barns at a height greater than 2 m with their entrance hole not facing the prevailing winds, and each box was separated at a distance of a least 5 m.

A total of 17 Starling nestlings from 6 nests and 74 Myna nestlings from 21 nests were measured from November to mid-March. According to Heather and Robertson (2000), the main nesting period for Starlings in NZ is from October to the end of January and the main nesting period for Mynas is from mid-October to mid-March. Data I collected were therefore only from second broods, or re-nesting attempts of Starlings, while data for the Myna contained both first and second broods, to ensure both species were being studied at the same time of the year. A nested ANOVA indicated that there were no differences in FA between the two broods of mynas ($p < 0.05$) and therefore the data were pooled.

The nestlings were temporarily removed from their nest boxes and each individual underwent three repeated measurements of bill length (distance from front of each of the nares to tip of bill), tarsus length (from intertarsal joint to distal end of tarsometatarsus), and unflattened wing chord length (from radial carpal joint to tip of longest primary feather of closed wing, or from the radial carpal joint to the tip of the phalanges on nestlings in which feather development had not yet occurred; Fig. 3.1).

Bill and tarsus lengths were measured using digital calipers (Mitutoyo, Mitutoyo Corporation, Japan) to the nearest 0.01 mm, and the wing chord was measured using a stopped rule to the nearest 0.5 mm. All measurements were taken by only one person (C.A. Debruyne) to reduce observer differences or measurement biases. Each nestling was measured every three days (from day/age 2 after hatching onwards; day zero = hatching) until they fledged the nest.

Measurement error (ME), the removal of outliers, and testing for the presence of Directional Asymmetry (DA) and antisymmetry were calculated according to Palmer (1994) and Palmer and Strobeck (2003). As suggested, a series of scatterplots of trait measurements followed by Grubb's Tests (Sokal and Rohlf 1995) were conducted to remove any anomalous individuals. To determine if ME was negligible compared to FA, two-way mixed model ANOVAs were conducted for every trait and age group separately using the measurements of each side (as a fixed factor), and individuals (as a random factor). In addition, DA and antisymmetry were tested by calculating the skew and kurtosis of the frequency distributions of the between-sides measurements along with t-tests (comparing the mean [right minus left] to zero) for every trait and age group examined. The two-way mixed model ANOVA indicated that Day 11 bill length measurements did not meet the criterion for FA (there was no significant difference between ME and FA $p = 0.76$) and therefore was omitted from further analyses.

FA indices and statistical analyses

Detailed definitions of the FA indices are found in chapter 1. As with earlier studies of FA in adults, I measured chick FA using FA1 (defined as the absolute asymmetry [right – left]) and FA8a (defined as the absolute relative asymmetry

[ln(right - left)], the size-scaled estimate of between-sides variance). Both FA1 and FA8a were calculated for each age/day group for both Mynas and Starlings according to Palmer and Strobeck (2003). As FA8a differed significantly between traits ($p < 0.05$ for all traits) during the calculation procedure of the various FA indices, it was only used for between-species and between-trait comparisons of FA throughout the nestling period. In addition, FA1 was used for within-trait examination of FA patterns throughout the growth period. Differences in FA between species were examined using general linear mixed-effects models for each trait and were conducted with FA8a as the dependent variable, species and nestling period (calculated by dividing the age/day by total mean nestling period) as explanatory variables, and individual nestlings within each nest as a random nested variable. To examine how traits differed in FA throughout the nesting period a general linear mixed-effects model was conducted with FA8a as the dependent variable, trait and nesting period as explanatory variables, and individual nestlings within each nest as a random nested variable. In addition, to examine the within-trait pattern of FA during the nestling period, general linear mixed-effect models were performed using FA1 as the dependent variable, and individual nestlings within each nest as a random nested variable for each trait. A general linear mixed-effect model was also conducted using FA8a as the dependent variable, trait as the explanatory variable, and individual nestlings within each nest as a random nested variable to determine if FA levels differed between traits on the final day of measurement (Day 20) in Mynas only, due to the small sample size in Starlings. The random nested variable was utilized in all mixed-effects models to avoid pseudoreplication, and the dependent variable was square-root transformed prior to all analyses to meet the assumptions of normality and homoscedasticity of variances (Sokal and Rohlf 1995). Each model was fitted (with

main effects and interactions), and explanatory variables were removed one at a time, using a stepwise deletion method, based on $p < 0.05$ (Crawley 2007). The backwards elimination of variables continued until a minimum adequate model (where all retained variables are significant) was obtained.

All tests to obtain the necessary FA values were performed using SPSS (Version 11.04, SPSS Inc. 2005), with a significance level of $p < 0.05$. In addition, the general linear mixed-effects models were carried out using R (Version 2.6.2; R Development Core Team 2008) with the NLME package.

Results

The mixed-effects models revealed no significant differences in levels of FA between species in any of the traits examined ($p > 0.05$ in all cases; Table 3.1). However, the interaction between species and nestling period for wing chord tended towards significance (Table 3.1), with higher FA levels in Starlings until Day 7, which then decreased to a level lower than Mynas until their final day of measurement on Day 17. Due to the lack of significant difference in FA between species throughout the growth period, both species were pooled for the remainder of the analyses.

In contrast to the lack of differences between species, the final models using both species combined indicated that there were significant differences in FA throughout the nestling period. FA8a decreased as the nesting period progressed for all traits (Table 3.2), which suggests that FA decreased as the trait reached its maximum size. Absolute FA (FA1) also differed significantly during the growth period for all traits, and peaks in FA were observed in the wing chord and tarsus at

approximately day 14 of the nestling period (Table 3.2; Fig. 3.2 a, b), whereas bill length appeared to have increased linearly (Fig. 3.2 c). Moreover, traits differed in the level of FA they exhibited throughout the nesting period. There was no difference between tarsus and bill length FA, however wing chord FA differed significantly from the other traits, and had the most pronounced decrease throughout the nesting period (estimate $\pm$ SE = -0.087 $\pm$ 0.013, $p < 0.001$; Fig. 3.3). Lastly in Mynas, traits differed in their levels of FA on the final day of measurement (tarsus: estimate $\pm$ SE = -0.059 $\pm$ 0.013, $p < 0.001$; wing chord: estimate $\pm$ SE = -0.083 $\pm$ 0.013, $p < 0.001$; Fig. 3.4) suggesting that although FA decreased by the end of the growth period, FA did not equalize among traits before fledging.

Discussion

Teather (1996) noted that few studies have been conducted examining changes in the FA of morphological traits throughout the developmental period of an animal. Moreover, no studies have compared potential differences in the ontogeny of FA between two related species. In this study, I examined FA in the nestlings of two species, Starlings and Mynas, which were both introduced to NZ in the 19th century and are now abundant, but which had experienced differences in the level of bottleneck severity during their establishment. However, I found no significant differences in FA between the two species even though it was predicted that Mynas would have greater FA since they passed through a more severe bottleneck (indeed, they had passed through two bottlenecks as the NZ population was sourced from Australia, and the species passed through a bottleneck when first established in that country). In contrast, FA did change throughout the course of the nestling period (in

both species), and some of the traits followed a similar parabolic trend as demonstrated in previous studies with other species. FA8a was negatively correlated with nestling period for all traits but was not equal on the final day of measurement.

Despite finding no significant differences in FA between the two species, it is not entirely clear if this indicates a lack of a bottleneck effect or if such an outcome has been masked by other confounding variables. Furthermore, I have assumed that the differences in bottleneck size between the two species have lead to differences in the current level of genetic variation present. This may not be the case as population bottlenecks do not necessarily have strong effects on genetic variability if there is a rapid increase in population size after the bottleneck event (Frankham et al. 2002), which was the case with both Starlings and Mynas. Early studies by Baker and Moeed (1987) and Ross (1983) also both found that observed levels of heterozygosity remained unchanged in introduced NZ Myna and Starling populations compared to their source populations, which would support the lack of a difference in FA between the two species. However, genetic analyses have vastly increased in accuracy since these early analyses and further genetic studies are now needed to determine exactly how differences in the size of bottlenecks faced by these two species have affected their current levels of genetic diversity.

Although bottleneck severity and its potential genetic consequences did not appear to have strong influences on the levels of FA in my study, one would still expect some differences in FA levels between species due to differences in environmental stress. For example, several studies have indicated that Mynas are dominant in encounters between the two species (Counsilman 1974; Pell and Tidemann 1997), which might subject Starlings to greater levels of stress. On my study area, I also noticed that Mynas seemed to out-compete Starlings for nest boxes

and displaced Starlings from feeding habitats, both of which may have increased stress on Starlings and lead to higher FA. Thus it is possible the higher FA levels I found in Starling wing chords may have been a result of this species experiencing higher level of environmental stress compared to the Mynas.

In contrast to starlings, Mynas may have been under less stress as they benefited from increased food availability through predation of Starling nestlings, and acquiring optimal nesting and feeding territories through greater dominance. Greico (2003) found reduced tarsus asymmetry in nestling Blue Tits (*Parus caeruleus*) supplemented with food and suggested that FA may depend on energy intake during development. Conversely, Hovorka and Robertson (2000) found no evidence of increased asymmetry in food-deprived nestling Tree Swallows, and they suggested this was due to strong stabilizing selection for wing symmetry since they are a migratory and aerially insectivorous species. I was unable to study the effects of food availability or Starling nest predation by Mynas, and the role these may have played in FA expression. However, further analyses are clearly required to demonstrate possible relationships between FA and whether differences in the level of stress experienced by each species might mask any effects on FA due to bottleneck size.

Ectoparasite loads have also been known to affect FA in nestling birds. For example, Bize et al. (2004) found higher FA in the wing chord of Alpine Swift (*Apus melba*) nestlings with experimentally elevated ectoparasite loads and suggested that it might have been a result of a depletion or re-allocation of energy required for growth. Similarly, Brown and Brown (2002) found reduced wing and tail FA in fledged juvenile Cliff Swallows (*Petrechelidon pyrrhonota*) that originated from experimentally fumigated colonies. A concurrent study of ectoparasite loads in my study populations found that Starling nestlings had higher parasite loads than Mynas

during the early nestling period (Allen 2008 PhD thesis, unpubl. data). Elevated parasite loads are likely to increase stress and may explain the slightly higher FA levels in Starlings. As with other environmental stresses, it is possible that a disproportionately greater level of stress on Starlings due to greater parasite loads, might result in a similar level of FA between the species despite the differences in bottleneck size.

Aparicio (2001) noted that FA levels might be variable among traits due to differences in the energetic costs of growth during development which may explain my observed differences between the traits. Moreover, Swaddle and Witter (1997) suggested that compensational growth mechanisms could stabilise FA levels as the trait develops. The parabolic pattern of absolute FA (FA1) displayed by the wing chord and tarsus during the nestling period also coincides with Aparicio (1998, 2001) FA-growth model in which FA decreases as the trait reaches its maximum size. A possible explanation for the linear increase in FA of the bill length may be because it has not yet reached its maximum size before leaving the nest and therefore the parabolic trend is not apparent. This result would not be too surprising as it is well known that different parts of the body can develop at different rates (Ricklefs 1998).

My measurements of relative FA indicated that FA of the wing chord was lowest of all of the traits on the last day of measurement, which is in agreement with the suggestion of Swaddle and Witter (1997) that FA should be lower in traits that are necessary for survival. Wing traits are important for survival since for most birds it is their primary method of locomotion, allowing for prompt escape from predators, access to remote feeding areas, and higher quality habitats through migration and dispersal (Norberg 1979; Pennycuick 1989). Moreover, Swaddle (1997) found that an increase in wing asymmetry decreased flight performance in Starlings and stressed the

importance of considering biomechanical performance when studying DI. Ground locomotion may be used with different foraging strategies (Grant 1966; Fretwell 1969; Grant 1971) and both Starlings and Mynas are primarily ground feeders (Faere and Craig 1999). This would indicate that tarsus symmetry may also be important for survival. On the other hand, both species are also opportunistic feeders and have a wide dietary range (Faere and Craig 1999) so perhaps the bill may be of less functional importance in feeding compared to traits associated with locomotion. As a result, there might be a strong stabilizing selection for symmetry in wing chord, and tarsus, and less for the bill. However, the exact fitness consequences of FA in each of these traits is speculative at present, and estimates of the strength of selection against high levels of FA need to be made before it will be possible to rank the importance of each individual trait.

In summary, differences in bottleneck severity between Mynas and Starlings did not have an apparent affect on FA in their nestlings, at least for the morphological traits I measured. However, my results suggest that other environmental stressors, such as food availability and habitat competition might also influence FA and if these affect Mynas and Starlings differently, then detecting effects due to bottleneck size alone may be difficult. The patterns of asymmetry during growth I found were similar to that observed in other studies. Differences in levels of FA between traits were also found and were most likely due to the differing energetic costs of development and the functional importance of the trait. These findings may provide a better understanding of developmental FA and the importance of trait choice when studying DI. Future studies using cross-fostering techniques or controlled lab experiments, and blood sampling to control for possible maternal and environmental confounds that could be masking potential bottleneck effects, along with an

assessment of the energetic costs of trait development and growth (such as food availability), could also be conducted which would allow for further understanding of the observed FA patterns in Starlings and Mynas.

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Figures

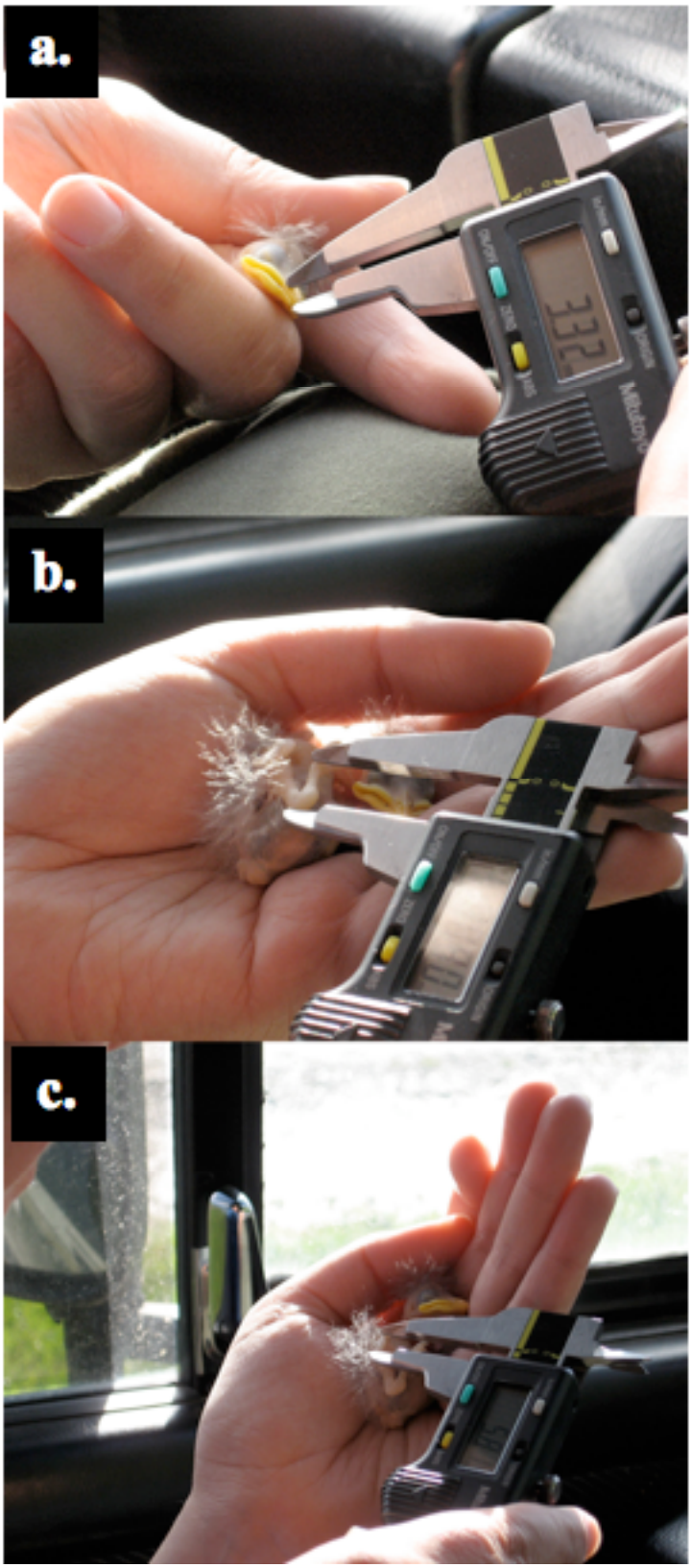
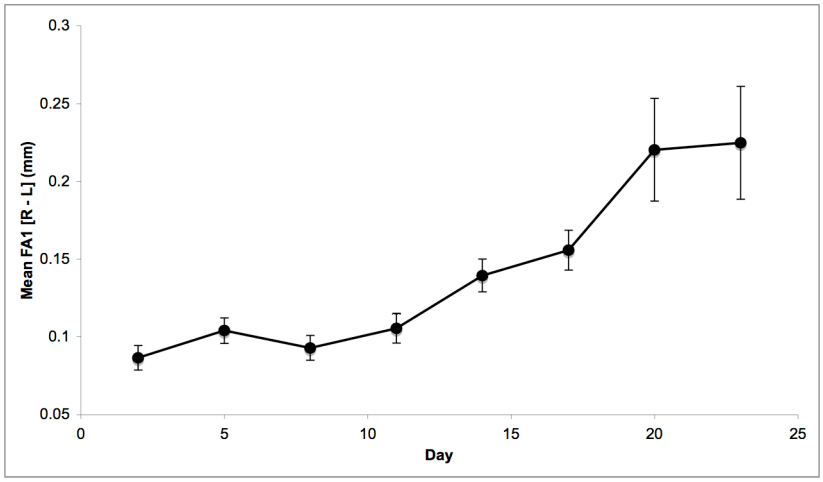
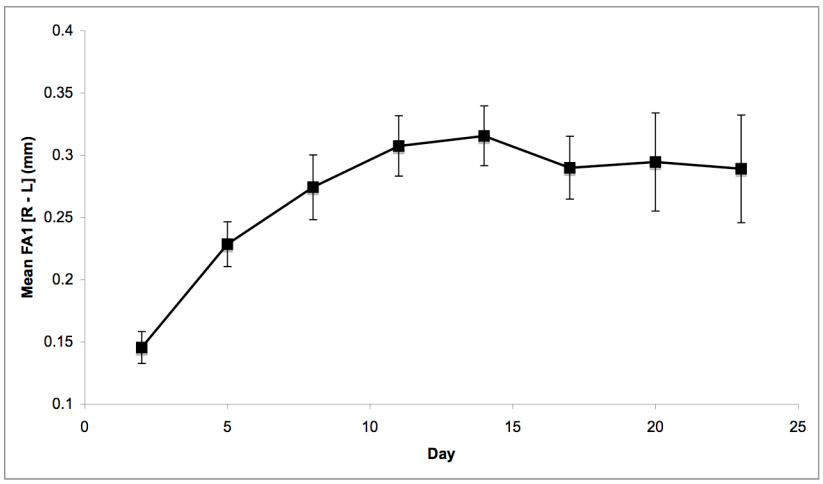


Figure 3.1. Measurements of the a. bill length, b. tarsus length, c. wing chord, of a five-day-old Starling nestling.

a.



b.



c.

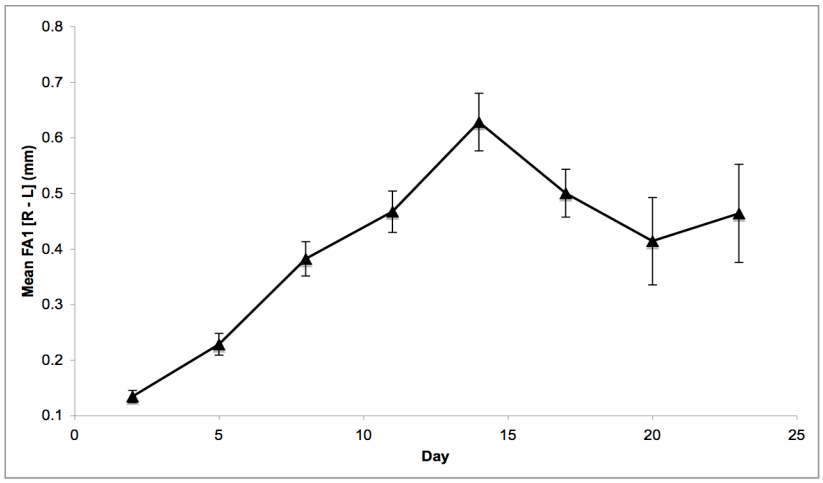


Figure 3.2. a-c. Mean ± SE absolute FA of the a. bill length, b. tarsus length, and c. wing chord of Myna nestlings during the developmental period.

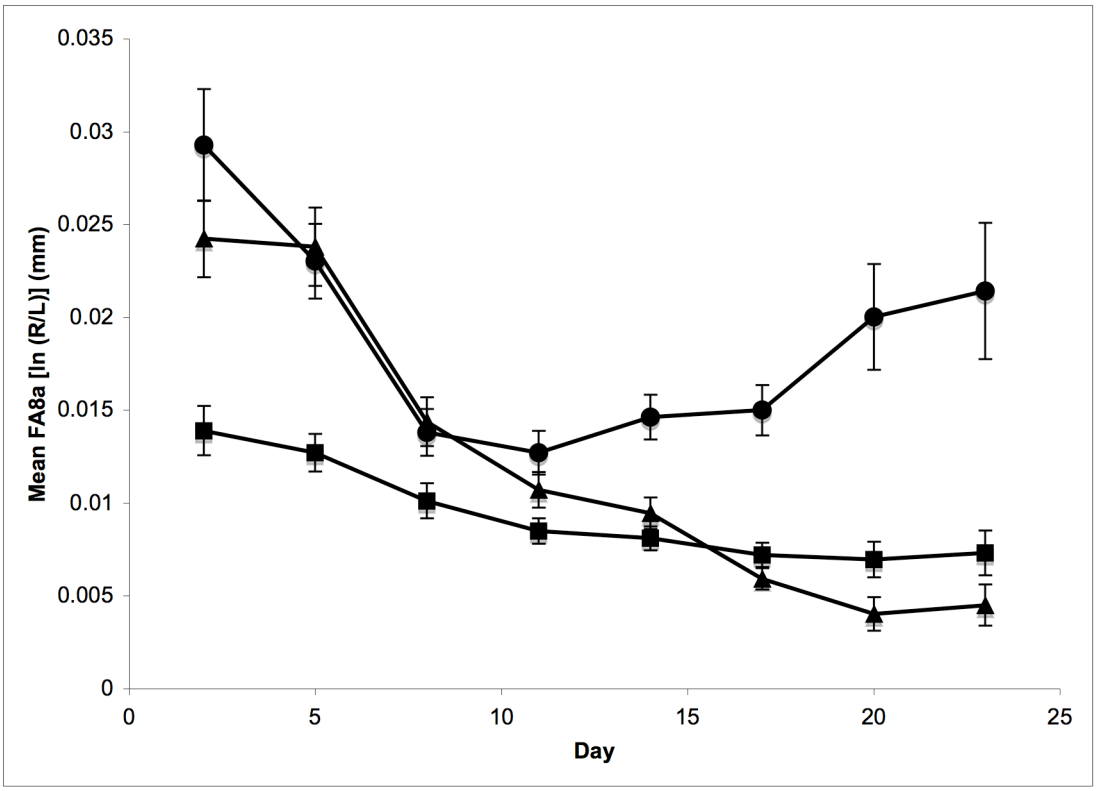


Figure 3.3. Mean ± SE of relative FA of the bill length (●), tarsus length (■), and wing chord (▲) of Myna nestlings during the developmental period.

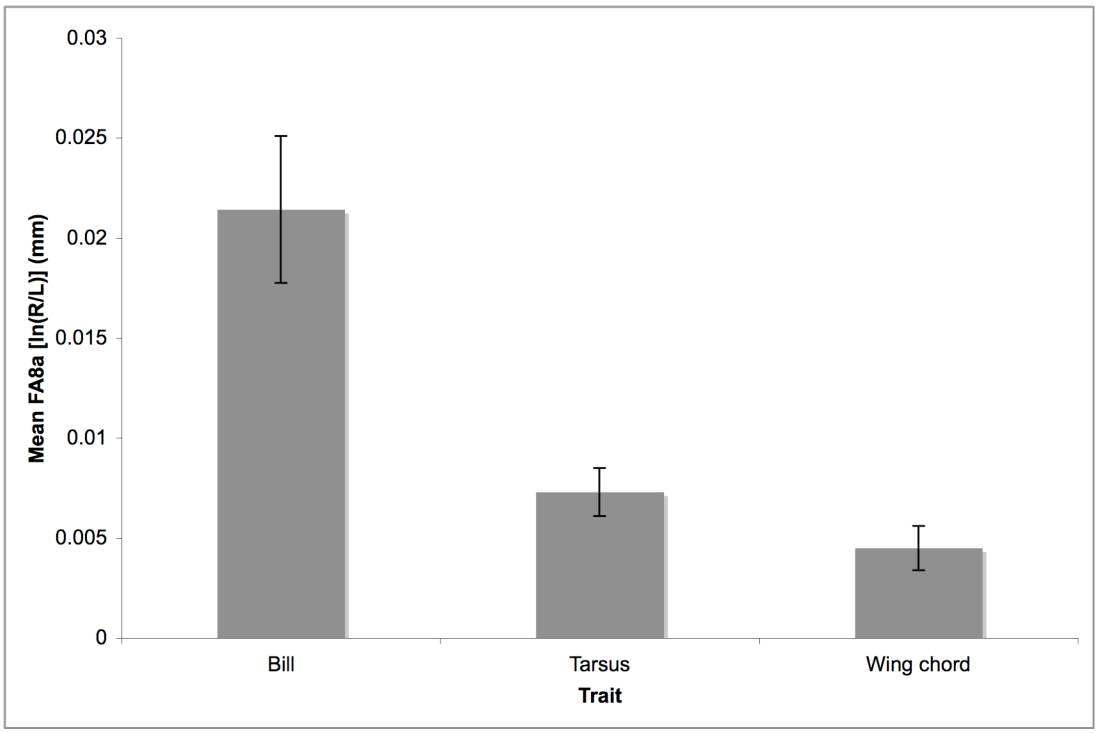


Figure 3.4. Mean relative trait FA of Myna nestlings on the last day of measurement (Day 23).

Tables

Table 3.1. Results from general linear mixed-effects models comparing relative FA in three morphological traits between Starling and Myna nestlings throughout the developmental period.

Trait	Predictor	Estimate	S.E.	P value
Bill length	Species	0.009	0.011	0.38
	Period	-0.037	0.010	< 0.01*
	Species × Period	-0.042	0.031	0.18
Tarsus length	Species	-0.005	0.005	0.31
	Period	-0.045	0.001	< 0.01*
	Species × Period	0.001	0.021	0.79
Wing chord	Species	0.009	0.007	0.25
	Period	-0.123	0.010	< 0.01*
	Species × Period	-0.047	0.028	0.09**

* Significant at $P < 0.05$
** Significant at $P < 0.1$

Table 3.2. Results from general linear mixed-effects models comparing levels of two FA indices across the nestling period. For each trait, age of the nestling is used as a predictor of FA in Starling and Myna nestlings.

Trait	FA indices	Estimate	S.E.	P value
Bill length	FA8a	-0.037	0.010	< 0.001
Tarsus length		-0.045	0.007	< 0.001
Wing chord		-0.123	0.010	< 0.001
Bill length	FA1	0.182	0.024	< 0.001
Tarsus length		0.164	0.035	< 0.001
Wing chord		0.359	0.053	< 0.001

CHAPTER 4: Comparative morphometrics of introduced birds in New Zealand

Abstract

The introduction of several avian species by acclimatization societies to New Zealand (NZ) from the United Kingdom (UK) over one hundred years ago has unintentionally created an ideal study system to examine potential changes in physical morphology due to bottleneck effects. In this study I compared differences in morphology between introduced NZ populations and their source populations in the UK in four external body traits of eight different species and compared this to the bottleneck size they passed through during their establishment. There were no significant differences in the extent of morphological change between introduced and source populations, nor among introduced species only in NZ, in relation to bottleneck size for any of the traits examined. However, overall changes in body size were observed in four species introduced to NZ: Blackbirds (*Turdus merula*), Greenfinches (*Carduelis chloris*), and Song Thrushes (*Turdus philomelos*) decreased in size, while Redpolls (*Carduelis flammea*) increased in size compared to their UK counterparts. Within-species comparisons revealed that all species exhibited some changes in trait morphology. Although these morphological changes do not appear to be related to bottleneck size, it is likely a combination of environmental and genetic factors has contributed to these differences and more work is needed to determine if the changes in morphology I observed are adaptations to the new environment in NZ.

Introduction

The introduction of new species into foreign locations, whether for intentional purposes such as species conservation or pest management, through self-introductions as a natural occurrence of dispersal, or by accident through trade and movement of people, is a common worldwide phenomenon. Upon European settlement of New Zealand (NZ) in the early 1800's, acclimatization societies were created to help settlers adapt to their new life by importing both flora and fauna from their homelands (Long 1981). More than 100 avian species were introduced into NZ of which about thirty species successfully established viable wild populations.

Most birds introduced to NZ were taken from source populations in the United Kingdom (UK). The numbers of individuals released per species varied from as few as a dozen up to approximately 1000 individuals (Thomson 1922). The founding of a new population from only a few individuals is termed a population bottleneck (i.e., a significant decrease in the number of individuals from the original population size), and it is known that severe bottlenecks can have various detrimental effects on individuals by lowering heterozygosity, increasing expression of deleterious genes in future generations, and by increasing the likelihood of inbreeding (Nei et al. 1974; Frankham et al. 2002). As a result, introduced species in NZ provide an ideal study system for comparing fitness between populations that have passed through bottlenecks of varying size to their source populations in the UK (Briskie 2006).

Although a population that passes through a severe bottleneck may manage to successfully establish itself in a new location, it is not always obvious whether such a population experiences negative fitness consequences as a result. For example, the European Starling (*Sturnus vulgaris*) is a well-known successful introduction from

Europe that now occurs in Africa, North America, and Australasia (Cabe 1993). This species has managed to establish populations worldwide (including NZ), even though each introduction forced populations through a bottleneck. It was probably because Starlings are highly adaptable due to their behavioural flexibility (Sol and Lefebvre 2000; Clergeau and Quenot 2007), and they take advantage of man-made structures and food resources that increase their reproductive success (Cabe 1993). On the other hand, Briskie and Mackintosh (2004) indicated that a potential fitness costs of severe bottlenecks in several introduced avian species into NZ, including the Starling, was an increase in hatching failure rates. Furthermore, morphological traits can also change after a population has passed through a bottleneck (e.g., Amiot et al. 2007). Some of these changes may adapt a population to its new surroundings, but as body morphology is a common indicator of fitness in many avian studies (e.g., Brown et al 1993; Murphy 2007; Hedenström 2008), changes in body morphology may reveal important biological changes a population may be experiencing.

The objective of my study is to compare the body morphology of introduced species in NZ to their UK source populations using a variety of morphological traits (e.g., body mass, bill, tarsus, and wing lengths). As source (and presumably non-bottlenecked) populations of species introduced to NZ are still extant in the UK, comparisons can be made between pre- and post-bottlenecked populations to examine if any morphological traits have changed, in which species such changes have occurred, and whether any changes in body morphology are a result of the size of the bottleneck that each species passed through during their establishment in NZ. Given the importance of bottlenecks to conservation management programs, my findings should provide an insight into the possible fitness consequences of introducing species into new habitats and whether this involves changes in body morphology.

Materials and Methods

Study Species and Study Areas

Body morphology data were taken from eight species of passerines (Table 4.1) at field sites in both the introduced NZ ranges of each species (Kaikoura, Ward, Blenheim), and in their source ranges in the UK (Rye Bay Ringing Observatory) from 2005-2006. The Kaikoura, Ward, and Blenheim study sites are situated along the eastern coast of the South Island in New Zealand (42°23' S 173°37', 41°48' S 174°06' E, 41°28' S 173° 57' E, respectively). These sites are predominantly agricultural areas, with patches of both introduced and native forest, providing suitable breeding and feeding habitats for the study species. Rye Bay Ringing Observatory is located in East Sussex, on the south east coast of the UK (50°54' N 0°41' E). This site is a wetland reserve which provides a stopover site for many migrating European warbler species, such as the Sedge Warbler (*Acrocephalus schoenobaenus*), and Reed Warbler (*Acrocephalus scirpaceus*). In addition, its forested surroundings, consisting mainly of native deciduous trees, and farmlands provides ample breeding and feeding habitats for the study species, allowing sufficient numbers to be captured for examining differences in body morphology. Although the exact location birds were captured for export is unknown, the south coast of England is the likely source of most of the birds exported to NZ in the 19th century, given this was the departure point for most ships to the southern hemisphere.

Field Procedures

A total of 555 birds from NZ and 530 UK were captured and measured. All birds were caught during the period from the end of their breeding seasons through to the end of autumn (February to mid-May in NZ; late August to early November in the UK) using mist nests with a mesh size of 30 mm and ground traps, according to the British Trust of Ornithology, and New Zealand standard protocols. Each individual was weighed to the nearest 0.1 g using digital scales. Measurements were then taken of bill length (from front of nares to tip of bill), tarsus length (from intertarsal joint to distal end of tarsometatarsus), and unflattened wing chord (from radial carpal joint to tip of longest primary feather of closed wing). These traits were chosen primarily due to their ease of measurement and their functional and physiological importance. The bill and tarsus lengths were measured using digital calipers (Mitutoyo, Mitutoyo Corporation, Japan) to the nearest 0.01 mm, and the wing chord was measured using a stopped rule to the nearest 0.5 mm. All measurements were taken by only one person (C.A. Debruyne) to eliminate observer differences or measurement biases. Wing chord data were only used from individuals which had already completed their post-juvenal or pre-basic moult to avoid the confounding effect of incomplete feather growth. Age and sex of each individual were determined by plumage characteristics (Svenson 1992). All birds were banded before release to avoid re-sampling the same individuals more than once. Lastly, individuals of each species were divided into two study groups according to location (either NZ or UK) in order to compare body morphology between introduced and source populations, respectively.

Statistical Analyses

Principal components analyses (PCA) were performed within species to test if each of the traits examined were correlated. Upon determination of correlation among traits, and due to the unbalanced design of the dataset, a general linear model (Sokal and Rohlf 1995) was preformed to determine if overall body size differed between the NZ and UK populations. In this analysis I used the correlated variable (PC1) from the PCA as the dependant variable, and location, age, and sex as independent variables. Multi-analysis of variances (MANOVAs) with type one error (Sokal and Rohlf 1995) were subsequently preformed to determine the trait differences for each species between the New Zealand and UK populations. For the MANOVAs I used each of the defining body traits (e.g., wing chord) as dependant variables, and sex, age, and location as independent variables. Two of the study species (Song Thrush and Dunnock) are sexually monotypic in size and plumage, and Redpolls are difficult to sex in their basic plumage (Svenson 1992). All individuals in these three species were pooled because sexual determination was unreliable and sex was removed as an independent variable in the multivariate analyses.

Bottleneck sizes for New Zealand populations were taken from the records of the acclimatization societies (Lever 1987; Table 4.1), and are based the sum of all birds released for each species with multiple introduction events but exclude introductions that are known to have failed. To examine the relationship between bottleneck severity and body morphology, least squares linear regressions were performed (on a trait-by-trait basis) using mean percent change as the dependant variable, and bottleneck size as the independent variable. This was repeated on every age and sex group of each species, respectively. All tests were performed using SPSS (Version 11.04, SPSS Inc. 2005), with a significance level of $p < 0.05$, however,

significance of the MANOVAs were determined using a Bernoulli process which calculates the probability of only one test of being statistically significant due to chance alone (Moran 2003). I also used the comparative analysis by independent contrasts software (CAIC) to test for potential phylogenetic artefacts in these regression analyses (Purvis and Rambaut 1994).

Results

PCA analyses revealed strong correlations between all of the traits for all species (PC1 > 40% of total variance, eigenvalue > 1.00; Table 4.2), suggesting that each trait changed proportionately in size with all of the other traits. Subsequent multivariate analyses indicated significant differences in overall body size between the NZ and UK populations in four out of the eight species examined (Blackbird: $F_{10,107} = 3.38$, $p < 0.01$, n = 49 NZ, n = 69 UK; Greenfinch: $F_{8,124} = 4.17$, $p = 0.02$, n = 68 NZ, n = 65 UK; House Sparrow: $F_{10,137} = 4.05$, $p = 0.106$, n = 114 NZ, n = 22 UK; Redpoll: $F_{11,91} = 5.32$, $p = 0.03$, n = 54 NZ, n = 46 UK; Song Thrush: $F_{4,74} = 2.25$, $p = 0.01$, n = 26 NZ, n = 53 UK; Chaffinch: $F_{4,142} = 31.47$, $p = 0.19$, n = 107 NZ, n = 40 UK; Dunnock: $F_{5,162} = 3.83$, $p = 0.26$, n = 51 NZ, n = 117 UK; Goldfinch: $F_{5,139} = 4.95$, $p = 0.35$, n = 68 NZ, n = 77 UK). NZ populations of Blackbirds, Greenfinches, and Song Thrushes were significantly smaller than their UK counterparts but the reverse was true for redpolls which increased in size in NZ (Fig. 4.1).

An examination of changes in body morphology on a trait-by-trait basis indicated a number of significant differences in the size of each morphological trait between the NZ and UK populations in all species, however, some changes were dependant on age and sex (MANOVA; Table 4.3). Tarsal length was significantly

shorter in NZ populations of Blackbirds, Dunnocks, Greenfinches, Song Thrushes, and hatch year (HY) Redpolls (Fig 4.2), however it increased in after hatch year (AHY) Redpolls (Fig. 4.3). Wing chord length was shorter for NZ populations of Blackbirds, Greenfinches, Redpolls, and HY Song Thrushes (Fig 4.4), however it increased in AHY Song Thrushes (Fig. 4.5). Body mass was significantly higher for NZ populations of Chaffinches, Goldfinches, House Sparrows, and Redpolls (Fig 4.6). In contrast, NZ Blackbirds exhibited an overall decrease in mass, but NZ AHY males were lightest of all of the NZ age and sex groups (Fig 4.7). Lastly, only Redpolls showed a change in bill length, being longer in the NZ individuals (Fig 4.8). Using the Bernoulli process, the probability that these were significant due to chance alone was less than 0.001.

Linear regressions indicated that there were no significant relationships between bottleneck size and body size morphology among species between the UK and NZ populations in all of the traits examined ($p > 0.05$ in all cases; Table 4.4). These results did not change once I controlled for possible phylogenetic effects ($p > 0.47$ in all analyses).

Discussion

In my study I compared body size, and a variety of within-species morphological traits in relation to bottleneck severity between introduced species in NZ to their source populations in the UK. I found that body size in introduced NZ species has changed significantly from that observed today in their source populations in the UK – decreasing in several species and increasing in only one. Moreover, I found significant decreases in tarsal and wing lengths in some species, while mass and

bill lengths increased in other species. Although a variety of changes in body morphology have occurred, to some extent in all NZ populations, I found no evidence that any of these changes were a result of the size of bottleneck they passed through during their establishment in NZ. Thus it is not clear what has caused the morphological changes I observed and whether these changes are the result of adaptations to the NZ environment or due to drift that can occur when a population is founded by a small number of individuals. It is even possible the changes I observed are directly due to bottleneck effects but the small number of species I examined limits the power to detect strong linear effects of bottleneck size on trait expression.

Changes in trait size

More than half of my study species showed an overall increase in mass in the NZ populations relative to their UK source populations. Why should NZ birds be heavier? A number of studies have shown that an increase in body mass in birds increases predation risk (Lima 1986; McNamara and Houston 1987; Gosler et al. 1995). This is because an increase in body mass may reduce the ability to make a quick escape from predators by decreasing flight manoeuvrability (Witter & Cuthill 1993; Metcalfe and Ure 1995; Bednekoff 1996; Kullberg et al 1996). On the other hand, an increase in body mass may be of benefit. Gosler (1996) found that individuals of greater body mass in Great Tits (*Parus major*) had an increase in over-wintering survival than their lighter counterparts. I cannot determine the reason for the observed increase in mass of most of the NZ populations of introduced birds, but it may be a result of a combination of changes in environmental factors. For example, an increase body mass may be due to fewer mammalian and avian predators in NZ than in the UK. There is no data to test this hypothesis although the lack of avian

predators on adults in NZ (e.g., one species of rare falcon) compared to the high number and diversity of avian predators on adults in the UK (e.g., several species of falcons and *Accipiter* hawks) suggest there may be lower overall predation pressure on birds in NZ. This could mean little penalty for an increase in size, especially if an increase in size leads to greater over-winter survival. Again, there is no data on survival rates of introduced species in NZ with which I could test this hypothesis and the reasons for the mass changes I observed clearly warrant further study.

Several species introduced to NZ showed significantly shorter wing lengths than their UK counterparts. A reduction in wing size could be attributed to a change in habitat and foraging tactics, since it has been shown to increase flight performance and manoeuvrability (Norberg 1979; Pennycuick 1989). In addition, wing length has been found to decrease in populations that have become sedentary (e.g., Perez-Tris and Telleria 2001). Rayner (1988) and Pennycuick (1989) both noted that longer wings reduce the amount of energy required in long-distant flights and since NZ populations of introduced birds are more-or-less sedentary compared to their UK counterparts (Higgins et al. 2006), this difference in migratory behaviour could explain the reduction in wing length in NZ. On the other hand, this hypothesis cannot explain the observed reduction in wing length of HY Song Thrushes in NZ but then an increase in the same trait in AHY individuals. Although sedentary, there may be other environmental factors, such as age-related predation pressures on NZ Song Thrushes that have not been accounted for, and further analyses are required to explain my observed findings.

Changes in tarsus length of birds has likewise been linked with their foraging tactics – birds with longer tarsi forage on more stable perches than individuals with shorter tarsi (Grant 1966; Fretwell 1969; Grant 1971). Therefore, the observed

reduction in tarsus length of NZ populations of some species could also be a result of a change in foraging tactics or how changes in habitat structure have altered foraging in NZ birds, such as through a decrease in the amount of stable, or larger-branched, perches. Such changes would also need to be age-related to account for age differences in Redpolls tarsal lengths. Unfortunately, examination of possible changes in foraging tactics of introduced birds has not been of yet performed and at present I cannot explain why tarsus length has changed in some birds introduced to NZ.

Redpolls were the only introduced species in NZ to have shown an increase in bill length. Changes in bill length are often associated with changes in feeding habitats (Grant 1968). Larger-billed birds feed on larger food items (Bowman 1961; Hespenheide 1966; Pulliam and Enders 1971). Since there have not been any comparative studies on possible changes in diet in adult introduced birds in NZ, it can only be hypothesized that NZ Redpolls have undergone a possible alteration in diet to suit the variety of seeds available. One might expect redpolls are exploiting larger seeds in NZ compared to the UK and this has resulted in the adaptation of longer bills, but at present this idea cannot be tested.

Changes in body morphology

Body size is known to vary geographically – with individuals being larger in size in cold climates and smaller in warmer climates (Bergmann's Rule; Mayr 1970). The observed decrease in body size in Blackbirds, Greenfinches, and Song Thrushes may be a result of differences between NZ and UK climates. MacLeod et al. (2008) found milder temperatures, less seasonality, and fewer extreme weather conditions in NZ which support Bergmann's Rule for a decrease in body size in NZ birds, however,

it does not account for the increase in body size in Redpolls, and the un-changed body size of the remaining introduced species. Clearly, there must be other possible reasons that could account for these changes. Although there were no correlations between any morphological traits and the size of the bottleneck each species passed through, it is possible that NZ populations may be experiencing different selection pressures as observed by changes in trait morphology through differences in predator-prey interactions, habitat, and food availability. Consequently, introduced species may have undergone rapid adaptation in morphological traits to suit their new niches (Amiot et al. 2007). For example, rapid changes in bill morphology occurred in less than ten generations in introduced Red-whiskered Bulbuls on Réunion Island, which may have been a result of changes in diet (Amiot et al. 2007).

Changes in body morphology may have also been a result of founder effects. Smaller, but heavier, individuals may have had greater chances of survival as they were transported in small cages for several months at sea. This could have resulted in a "smaller-sized" founding population due to selection against large individuals during the introduction process. In addition, founding populations could have been genetically limited for the expression of larger size traits. Baker (1980) examined morphometric differentiation in House Sparrows at various localities within NZ and found a reduction in morphometric variation compared to introduced populations in North America and ancestral European lineages possibly due to genetically restricted founding stock and environmental homogeneity. Since NZ founding populations were introduced in small numbers, individuals captured and brought over may have been of smaller body size than the average source population resulting in smaller NZ individuals.

Lastly, random genetic drift could account for the observed changes in body morphology. Baker et al. (1990) attributed changes in morphology of skeletal characters of introduced Chaffinches in various locals within NZ to random drift since he found no evidence of environmental adaptation. On the other hand, Rice and Hostert (1993) and Clegg et al. (2002) both noted that changes in body morphology could not solely be a result of drift due to the small introductory time frame in which they usually occur. Moreover, Clegg et al. (2002) found that observed changes in trait size of Silvereyes (*Zosterops lateralis*) are most likely due to directional selection since shifts body size appeared to be non-random. It was beyond the scope of my study to measure the fitness consequences of the changes in morphological traits I observed, but it should be possible to rule out random drift as an explanation by measuring the fitness consequences of changes in morphology and whether there is any evidence such changes are due to directional selection.

In summary, I found that changes in body morphology have occurred in all of the introduced species I examined, with most birds decreasing in body size, yet increasing in mass. However, these changes do not appear to be related to the size of the bottleneck in which they passed through, but may be a result of a combination of environmental factors and genetic constraints. Conservation management practices should take note of the potential for translocated populations of native and endangered species to undergo rapid changes in body morphology. Whether these changes are the result of adaptations to local habitats, and thus to be encouraged, or whether they are an unintended outcome of passing through a bottleneck, and thus a worry, are not clear. Determining the exact reasons for morphological changes in translocated populations is now needed in order to determine whether such changes have implications when dealing with endangered species.

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Figures

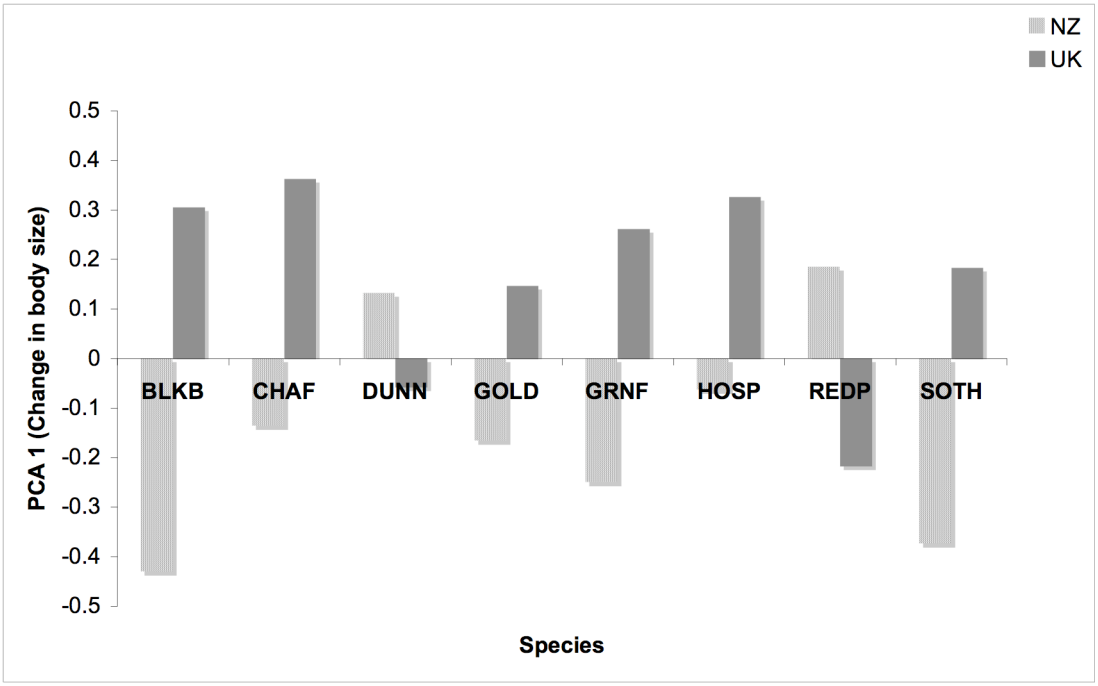

Figure 4.1. Change in body size between NZ and UK population of each species. An increase in PCA 1 indicates an increase in body size. BLKB = Blackbird, CHAF = Chaffinch, DUNN = Dunnock, GOLD = Goldfinch, GRNF = Greenfinch, HOSP = House Sparrow, REDP = Redpoll, SOTH = Song Thrush.

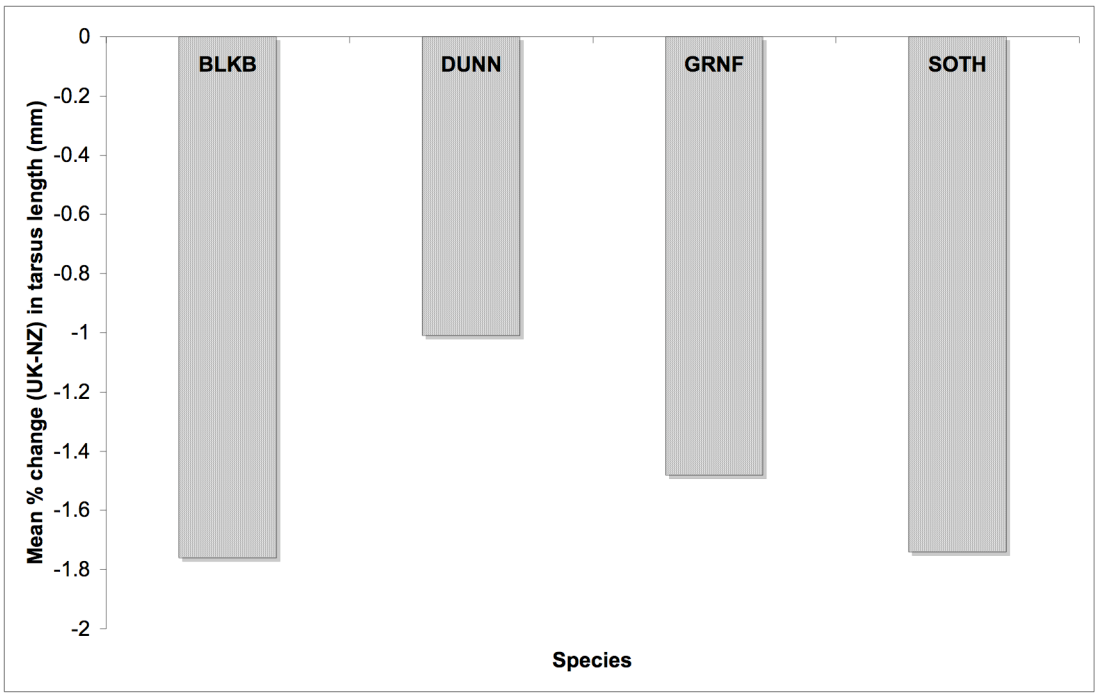

Figure 4.2. Changes in tarsus length between NZ and UK populations of each species. Only species with significant differences between the two countries are plotted. BLKB = Blackbird, DUNN = Dunnock, GRNF = Greenfinch, SOTH = Song Thrush.

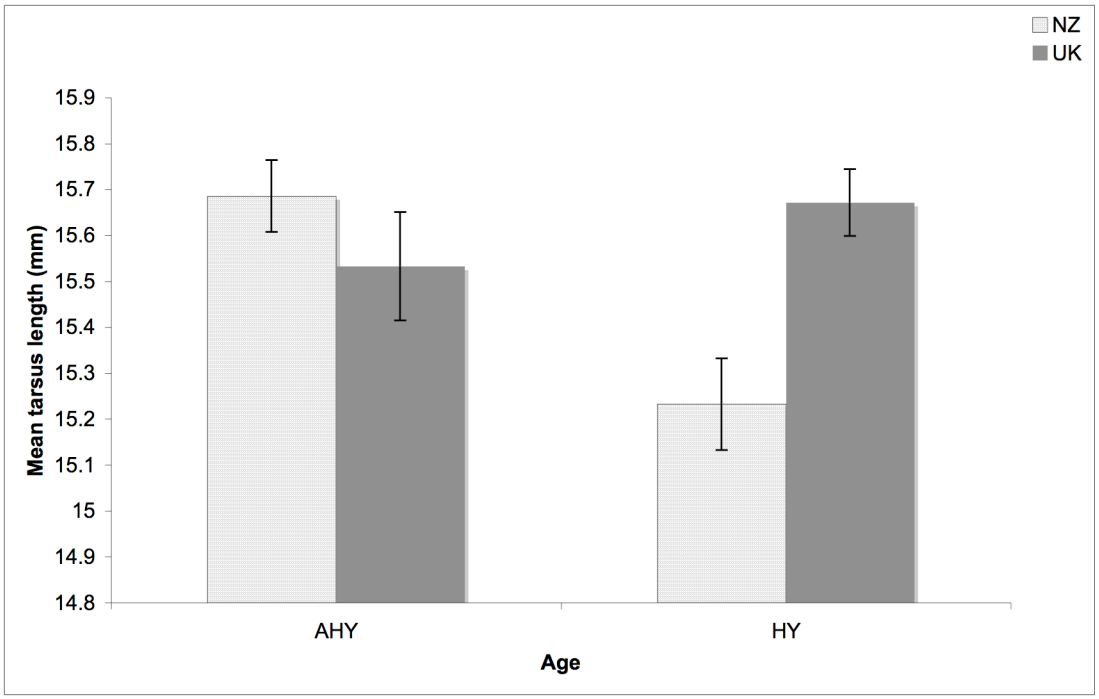

Figure 4.3. Mean (±SE) tarsus length of redpolls in the NZ and UK populations of this species. Tarsus lengths are given for both after hatch year (AHY) and hatch year (HY) birds.

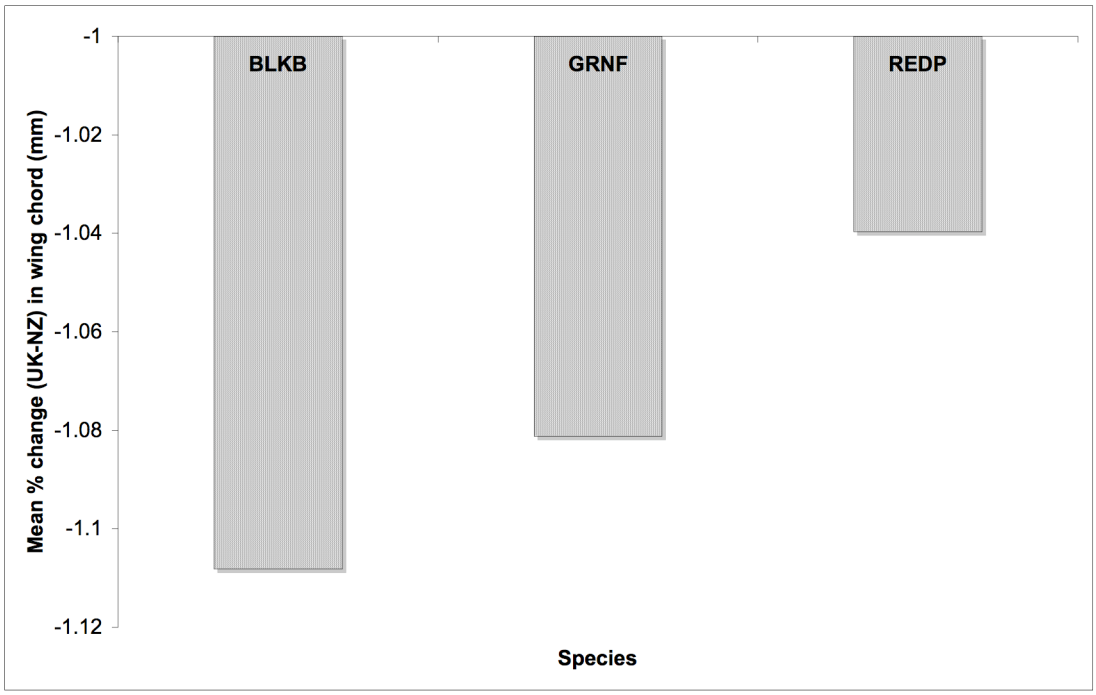

Figure 4.4. Changes in wing chord length between NZ and UK populations of each species. Only species with significant differences in wing chord length between the two countries are plotted. BLKB = Blackbird, GRNF = Greenfinch, REDP = Redpoll.

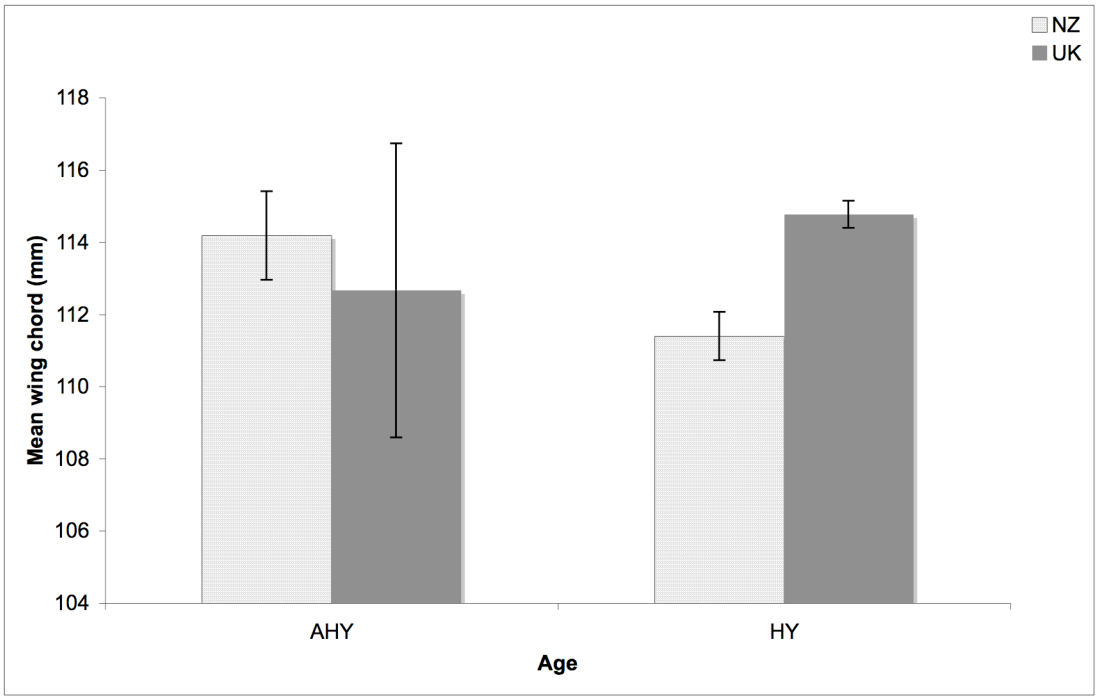

Figure 4.5. Mean (±SE) length of the wing chord in NZ and UK populations of the song thrush. Wing chord lengths are plotted separately for after hatch year (AHY) and hatch year (HY) birds.

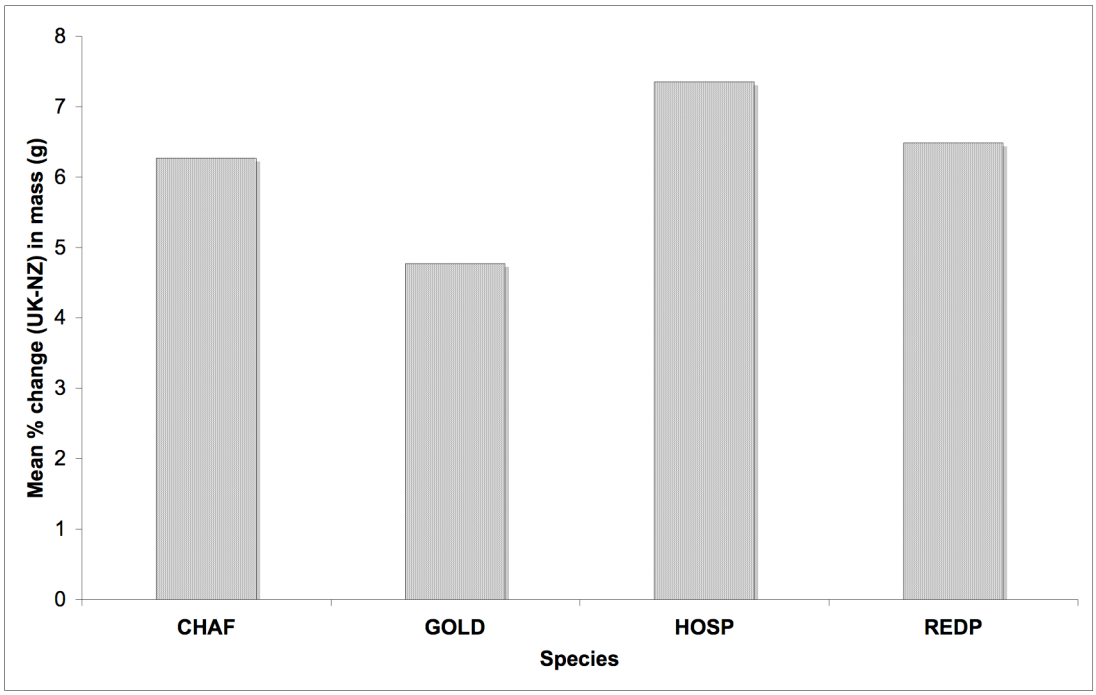

Figure 4.6. Changes in mass between NZ and UK populations of each species. Only species with significant differences in body mass between the two countries are plotted. CHAF = Chaffinch, GOLD = Goldfinch, HOSP = House Sparrow, REDP = Redpoll.

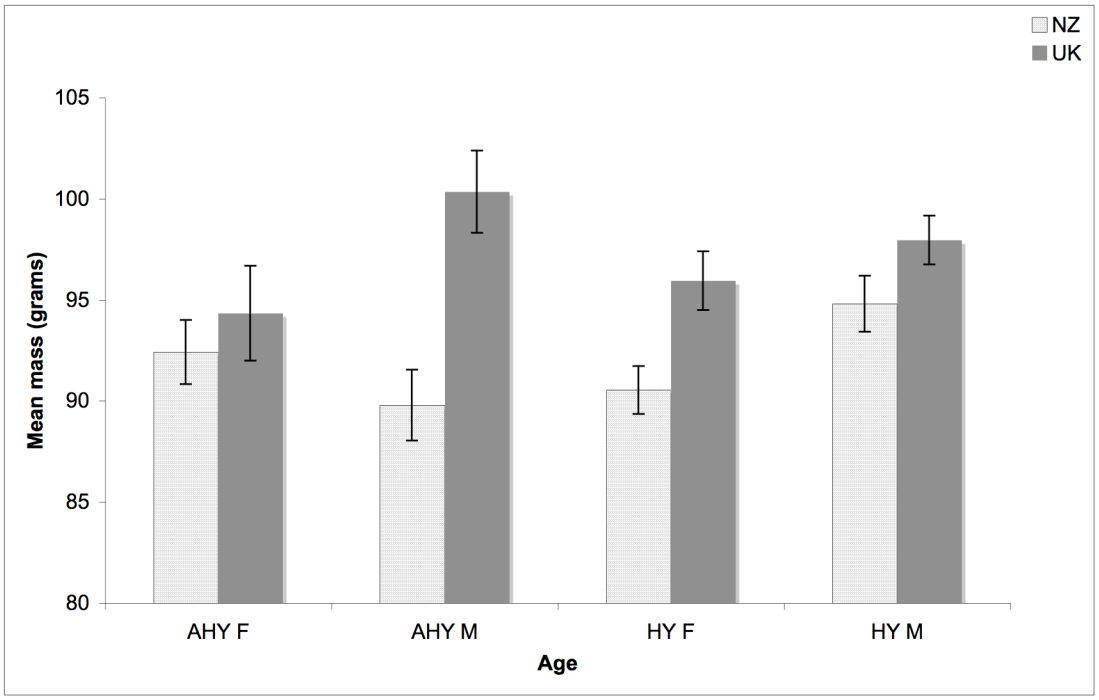

Figure 4.7. Mean (±SE) mass of NZ and UK populations of the blackbird. The mass of female (F) and male (M), and after hatch year (AHY) and hatch year (HY) blackbirds are plotted separately.

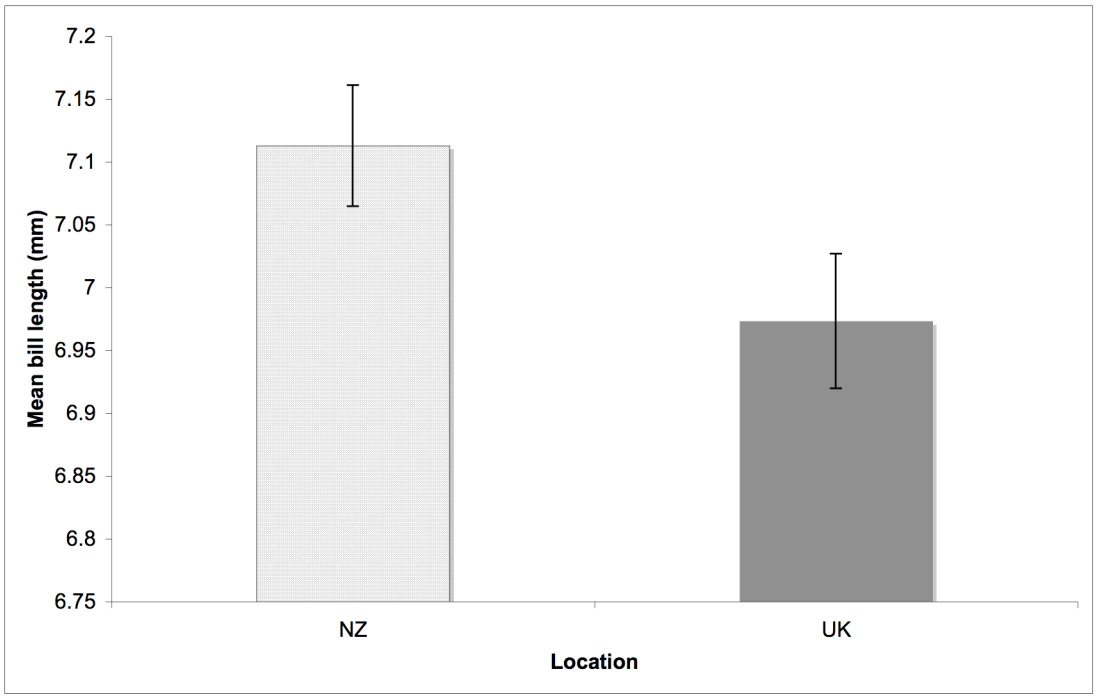

Figure 4.8. Figure 4.7. Mean (±SE) bill length of NZ and UK populations of Redpolls.

Tables

Table 4.1. Number of individuals measured for each species from their source populations in the United Kingdom (UK) and their introduced ranges in New Zealand (NZ). The size of bottleneck (number of individuals introduced) for each species in NZ is also given.

Species	***n***		**Bottleneck Size**
	UK	**NZ**	
Greenfinch *Carduelis chloris*	72	68	66
House Sparrow *Passer domesticus*	27	125	111
Dunnock *Prunella modularis*	117	53	284
Chaffinch *Fringilla coelebs*	44	108	377
Song Thrush *Turdus philomelos*	57	25	474
Goldfinch *Carduelis carduelis*	88	69	516
Lesser Redpoll *Carduelis flammea*	50	57	599
Blackbird *Turdus merula*	75	50	808

Table. 4.2. PCA vector loadings of the mass, culmen length, tarsus length, and wingchord, for each species.

Species	Tarsus Length	Wingchord	Mass	Bill Length
Blackbird	0.782	0.468	0.855	0.251
Chaffinch	0.535	0.775	0.791	0.636
Dunnock	0.732	0.763	0.706	0.303
Goldfinch	0.675	0.761	0.796	0.655
Greenfinch	0.406	0.714	0.754	0.555
House Sparrow	0.738	0.462	0.713	0.603
Redpoll	0.662	0.542	0.797	0.640
Song Thrush	0.766	0.688	0.561	0.563

Table 4.3. MANOVAs indicating differences in trait sizes between NZ and UK populations. Significant differences are indicated in bold.

Species	**Trait**	**F**	**df**	**p**
Blackbird	Tarsus length	6.884	1,110	p = **0.010**
Chaffinch		1.770	1,142	p = 0.186
Dunnock		4.532	1,160	p = **0.034**
Goldfinch		10.505	1,122	p = 0.708
Greenfinch		7.273	1,117	p = **0.008**
House Sparrow		0.780	1,134	p = 0.379
Redpoll		4.431	1,102	**p = 0.003***
Song Thrush		10.287	1,77	p = **0.002**
Blackbird	Wingchord	7.324	1,110	p = **0.008**
Chaffinch		1.959	1,142	p = 0.163
Dunnock		0.373	1,160	p = 0.543
Goldfinch		2.370	1,122	p = 0.124
Greenfinch		4.377	1,117	**p = 0.038**
House Sparrow		0.034	1,134	p = 0.854
Redpoll		5.307	1,102	p = **0.024**
Song Thrush		5.130	1,77	p = **0.026***
Blackbird	Mass	19.614	1,110	p = **0.029****
Chaffinch		8.219	1,142	p = **0.004**
Dunnock		0.010	1,160	p = 0.922
Goldfinch		10.505	1,122	p = **0.002**
Greenfinch		2.184	1,117	p = 0.270
House Sparrow		14.495	1,134	p = **0.001**
Redpoll		17.459	1,102	p > **0.001**
Song Thrush		0.143	1,77	p = 0.707
Blackbird	Bill length	2.239	1,110	p = 0.409
Chaffinch		0.405	1,142	p = 0.525
Dunnock		0.653	1,160	p = 0.423
Goldfinch		0.296	1,122	p = 0.585
Greenfinch		0.243	1,117	p = 0.404
House Sparrow		0.224	1,134	p = 0.112
Redpoll		4.052	1,102	p = **0.017**
Song Thrush		0.533	1,77	p = 0.468

* Dependent on either or sex groups

** Dependent of both age and sex groups

Table 4.4. Probability values of least squares linear regressions. Effects of bottleneck size on the differences in trait size between NZ and UK populations of different age and sex groups.

Age	**Sex**	**Tarsus Length**	**Wing Chord**	**Mass**	**Bill Length**
AHY	F	p = 0.776	p = 0.359	p = 0.590	p = 0.899
AHY	M	p = 0.848	p = 0.954	p = 0.346	p = 0.467
HY	F	p = 0.420	p = 0.447	p = 0.190	p = 0.106
HY	M	p = 0.777	p = 0.350	p = 0.494	p = 0.773
HY	UNK	p = 0.665	p = 0.206	p = 0.652	p = 0.348

CHAPTER 5: Population bottlenecks and physical abnormalities in introduced birds in New Zealand

Abstract

Physical abnormalities are generally rare in populations of wild animals, but are expected to be more frequent in species that have passed through severe genetic bottlenecks due to the increased expression of deleterious alleles with greater inbreeding. I compared rates of physical abnormalities among 11 species of European birds that were introduced to New Zealand in the 19th century, and had passed through bottlenecks of varying severity during their establishment, with that present in their source populations in the UK. A number of abnormalities were noted within both countries and included deformities of the feet, partial albinism and the production of excess tail feathers. Rates of abnormalities were slightly higher in NZ than in the UK, although this difference was not significant. There was also a slight increase in the rates of partial albinism with decreasing bottleneck size within NZ populations of introduced birds, but this trend was also not quite significant ($p = 0.06$). Overall, I found only weak support for increased rates of abnormalities among introduced populations of birds in NZ, although strong selection against abnormalities may make it difficult to detect severe abnormalities using current sampling techniques.

Introduction

Physical abnormalities may be caused by either a genetic mutation resulting in the aberrant phenotypic expression of a trait, or may be a consequence of exposure to environmental stressors that cause changes in their morphology. Abnormalities are usually a rare occurrence in wild avian populations since they are likely to decrease individual's level of fitness and survival (Ellegren et al. 1997). As a result, few studies have reported or examined frequencies of physical abnormalities, and most cases of abnormalities reported are from populations that are under high levels of environmental stress due to exposure to chemical contaminants (e.g., Møller 1993; Thompson et al. 2006; Mora et al. 2007).

In a large outbred population, physical abnormalities as a result of genetic mutations are likely to be rare due to compensation from the homologous normal allele. However, in species that pass through a severe population bottleneck (defined as a large decrease in the number of individuals from the original population size), inbreeding is likely to be more common and this can increase the levels of homozygosity and thus increase the chances that the same deleterious alleles are inherited from both parents (Frankham et al. 2002). Although not all abnormalities are necessarily due to an increase in the expression of deleterious alleles, if severe bottlenecks increase the levels of inbreeding, then one might expect a corresponding increase in the rates of abnormalities appearing in a post-bottlenecked population.

Severe population bottlenecks are becoming more common as ever increasing human-induced changes to the environment cause increasing numbers of species to become endangered. Physical abnormalities have been noted in a number of endangered species and it has been suggested these are due to the deleterious effects

of passing through a population bottleneck. For example, Dice Snakes (*Natrix tessellata*) in bottlenecked populations show higher incidences of scale deformities than outbred populations (Gautschi et al. 2002). Similarly, higher levels of testicular aplasia are found in inbred populations of Koalas (*Phascolarctos cinereus*; Seymour et al. 2001) and alleles for a lethal form of dwarfism are found in about 10% of surviving California Condors (*Gymnogyps californianus*), a highly endangered bird that passed through a bottleneck of just 28 individuals (Ralls et al. 2000).

Most reports of physical abnormalities in endangered animals are based on single case studies and often lack data from control populations with which to compare the "normal" levels of abnormalities. This is unavoidable in any project with an endangered species, as by definition, such species are already passing through a bottleneck. However, it may be possible to assess the effects of population bottlenecks by using introduced species as a surrogate model system. In the late 19th century, a number of species were introduced to New Zealand (NZ), primarily from the United Kingdom (UK). Today about 30 species of exotic birds are present in NZ, and as all still have relatively large and extant populations in their native range, one can compare the levels of abnormalities within each species, both before (levels in native range) and after (levels in NZ) passing through a bottleneck. As the number of individuals released in NZ also varied across species, from as few as a dozen Cirl Buntings (*Emberiza cirlus*) to as many as 800 Blackbirds (*Turdus merula*; Lever 1987), the effects of different sized bottlenecks on abnormality rate can also be assessed.

In this study I compared the proportion of physical abnormalities in the form of deformities and partial albinism between populations of introduced birds in NZ and their source populations in the UK. In addition, I examined if changes in the

proportion of physical abnormalities between NZ and UK populations were related to the size of the bottleneck each species past through. This study may reveal the importance of noting abnormalities in the management of endangered species as they may be an underlying indicator of the developmental stability of a population.

Materials and Methods

A total of 1485 individuals and skeletons from eleven species were examined for any signs of physical abnormalities (Table 5.1). This includes birds captured in the field and examined before release as well as an examination of skeletons prepared from a sub-sample of birds (see Chapter 1 for details on capture and measurement of live birds and Chapter 2 for details on skeleton preparation). Birds were assessed for the presence of abnormalities in both NZ (introduced populations) and in the UK (source populations). Abnormalities were categorized into two groups: physical deformities (including any previous sign of injury, aberrant bone growth, external growths, missing toes; Fig 5.1), and signs of partial albinism (having some lack of pigment in feathers).

Analysis of variance was performed to determine if there were any differences in the proportion of physical abnormalities between NZ and UK, using either the proportion of deformities, or proportion of partial albinism, as the dependant variable and location as the independent variable. To examine the relationship between bottleneck severity and physical abnormalities, least squares linear regressions were performed using the proportional change between NZ and UK, of either deformities or partial albinism, as the dependant variable, and bottleneck size as the independent variable (except for Cirl Buntings as there were no UK samples). Least squares linear

regressions were also performed to examine the relationship between bottleneck severity and physical abnormalities in species within just the NZ populations, using the proportional change of either deformities or partial albinism, as the dependant variable, and bottleneck size as the independent variable. I then used the comparative analysis by independent contrasts software (CAIC) to test for potential phylogenetic artefacts in these regression analyses (Purvis and Rambaut 1994). Bottleneck sizes for NZ populations were provided from the records of the acclimatization societies (Lever 1987; Table 5.1). All tests were performed SPSS (Version 11.04, SPSS Inc. 2005), with a significance level of $p < 0.05$.

Results

The types of physical abnormalities I observed included deformities in the foot, partial albinism and an increase in the number of tail feathers (Figure 5.1). ANOVAs examining differences in rates of abnormalities indicated that there was a significant difference between NZ and UK ($F_{1,19} = 4.52$, $p = 0.047$; Table 5.1), with NZ having a larger proportion of deformities than the UK. However, this significance was only obtained when I used one species (Rook) that had a sample size of only 13 birds in NZ and 16 birds in the UK. Upon removal of this species from the analysis, thus resulting in all remaining species having sample sizes of more than 20 individuals, the difference between NZ and the UK was no longer significant ($F_{1,17} = 2.67$, $p = 0.12$). There was also no significant difference in the rates of albinism between the two locations ($F_{1,19} = 0.43$, $p = 0.60$; Table 5.1). Linear regressions among species indicated that there were no significant relationships between bottleneck size and differences in the levels of physical abnormalities between the UK

and NZ (deformities: n = 10, r^2_{adj} = -0.12, p = 0.80; partial albinism: n = 10, r^2_{adj} = 0.33, p = 0.08). Likewise, within NZ only there was no significant relationship between bottleneck size and rates of either physical deformities (deformities: n = 11, r^2_{adj} = -0.53, p = 0.97) or rates of partial albinism partial albinism, although partial albinism tended towards significance (partial albinism: n = 11, r^2_{adj} = 0.34, p = 0.06). All of these regression analyses remained non-significant when potential phylogenetic effects were controlled (all p values > 0.39).

Discussion

I found only weak support for the prediction that there should be higher levels of physical abnormalities in the bottlenecked populations of species introduced to NZ in the 19th century. Although I noted a slightly greater frequency of abnormalities in NZ birds, such as deformed toes and feet, partial albinism, and the production of excess tail feathers, the rates of these abnormalities did not differ significantly from that I observed in the UK. There was also no strong relationship between the rates of abnormalities and the size of bottleneck each species passed through during their establishment, although the trend for partial albinism to increase in the more severely bottlenecked species was almost significant.

There are few estimates of rates of deformities in any study of wild birds, apart from those that have specifically looked at known environmental contaminants (e.g. Møller 1993; Thompson et al. 2006; Mora et al. 2007). One problem with estimating rates of abnormalities from a survey of wild birds is that any estimates obtained are likely to be biased. For example, abnormalities that reduce the survival of an individual are likely to disappear from the population relatively rapidly through

death and thus would not be included in a survey. Even if abnormalities do not cause immediate death, they might nonetheless change the behaviour of birds in such a way that it reduces the probability they are captured or encountered through sampling techniques such as mist-netting. One might be able to reduce this bias by focusing on nestling birds or earlier life stages of an animal (i.e., before such individual are removed by selection), although this would then miss any abnormalities that develop only in adults. Finally, the small rates of abnormalities typically observed in studies (e.g. 0-13% in my study) means that sample sizes need to be large enough to provide enough statistical power to detect differences between populations. With small sample sizes the inclusion of one or two additional abnormal individuals can greatly increase the calculated rate overall. Indeed, when I first compared the rates of abnormalities between NZ and the UK, I found a significant difference between the two countries, but this was due primarily to the small sample size of Rooks in my study, and this pattern disappeared when I only analysed species with a larger sample size.

Some physical abnormalities may not be due to direct effects of genetic mutations but instead the result of other causes. For example, it is possible that many of the deformities I noticed in the toes and feet were the result of avian pox, a disease which can disfigure bones after they have grown normally (e.g., Young and VanderWerf 2008). One could thus argue that any estimate of abnormal feet is really due to disease and not the genetic effects of severe population bottlenecks. However, as bottlenecks are expected to increase the susceptibility of a population to diseases and other pathogens, it may not matter from a management perspective as to the exact cause of the abnormality, only whether such abnormalities increase in endangered animals as a result of passing through a bottleneck. In other words, routine surveys of

levels of physical abnormalities may still provide useful information on the health and fitness of individuals in post-bottlenecked populations, even if the pathways by which such abnormalities arose may be a complex combination of several contributing factors.

I have assumed in this study that the possession of a physical abnormality generally has a fitness cost to the bearer. The types of abnormalities I noticed were relatively minor deviations from what would be considered a normal phenotype but I would argue that even these minor abnormalities should be deleterious. For example, deformities in the foot are likely to reduce the efficiency of perching, locomotion, and foraging (especially for species that use their feet for foraging; Dorcherty et al. 1991; Young and VanderWerf 2008). Abnormalities in plumage such as albinism could also affect survival by increasing conspicuousness to predators, and reduce reproduction through reduced mating success (Ellegren et al, 1997). Although it was beyond the scope of this study to measure the fitness costs of such abnormalities, it seems justified to consider them as detrimental to the individuals that possess them, and to conclude that elevated rates of abnormalities in a population of birds may indicate an underlying problem that is worthy of consideration by conservation biologists.

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Figures

Figure 5.1. Example of a physical deformity in a House Sparrow. Here the normal complement of 12 tail feathers is increased by an extra 4 feathers.

Tables

Table 5.1. Number of individuals examined for levels of physical abnormalities and the proportion of deformities and partial albinism for each species in both their source populations in the United Kingdom (UK) and their introduced ranges in New Zealand (NZ). The size of bottleneck (number of individuals introduced) for each species in NZ is also given.

Species	***n***		**Deformities**		**Albinism**		**Bottleneck**
	UK	**NZ**	**UK**	**NZ**	**UK**	**NZ**	**Size**
Cirl Bunting *Emberiza cirlus*	N/A	28	N/A	0.00	N/A	0.00	11
Greenfinch *Carduelis chloris*	87	93	0.02	0.04	0.02	0.00	66
House Sparrow *Passer domesticus*	44	165	0.05	0.05	0.00	0.01	111
Rook *Corvus frugilegus*	16	13	0.00	0.08	0.00	0.00	212
Dunnock *Prunella modularis*	145	71	0.03	0.13	0.00	0.00	284
Chaffinch *Fringilla coelebs*	45	137	0.04	0.04	0.00	0.01	377
Yellowhammer *Emberiza citrinella*	15	58	0.00	0.00	0.00	0.00	462
Song Thrush *Turdus philomelos*	61	29	0.02	0.07	0.00	0.03	474
Goldfinch *Carduelis carduelis*	117	71	0.01	0.01	0.00	0.00	516
Lesser Redpoll *Carduelis flammea*	50	78	0.00	0.09	0.00	0.00	599
Blackbird *Turdus merula*	92	70	0.04	0.04	0.03	0.06	808

GENERAL DISCUSSION

In this study I examined changes in a number of physical attributes of introduced birds using FA, comparative morphological measurements, and visual observations that may have potentially been a result of the size of the bottleneck each species passed through during their establishment in New Zealand (NZ). The only relationship I found in this study was a change in the levels of FA of external traits in relation to bottleneck severity. However, this relationship held only among NZ populations of introduced birds as I did not find that the levels of FA in NZ introduced populations were greater than in their source populations in the UK. Indeed, I found that some species had higher levels of FA in the UK than in NZ, a finding opposite to what was predicted. On the other hand, I did find that FA in external traits did vary among NZ populations in relation to bottleneck severity – FA varied in hatch year individuals for the wing chord length and in hatch year females for the ninth primary length, with those species passing through the most severe bottlenecks exhibiting higher levels of FA than species passing through larger bottlenecks. The resulting findings may be a subtle, but potential indicator of the negative consequences of severe population bottlenecks.

When I undertook comparisons of FA between NZ and UK on a species by species basis, I found that only FA of the tarsus length of Redpolls was higher in the UK population. Similarly, FA of osteological characters differed for only three species in two characters examined. FA of the tarsometatarsus length was higher in the UK source populations of Greenfinches and House Sparrows compared to their NZ counterparts, while the FA of the ulna was higher in introduced NZ Dunnocks. Reasons for this lack of generality of FA in both external and osteological characters

are unknown, however a combination of possible environmental and genetic stressors may have resulted in species- and character-specific FA relationships. Thus, my findings may indicate that only some traits are affected by DI while other traits appear to be relatively immune to stressors, at least those incurred by the range of bottleneck sizes I investigated.

I found no significant differences in nestling FA between Starlings and Mynas even though it was expected that Mynas would have greater FA since they passed through a more severe bottleneck (actually a double bottleneck if one also considers they passed through a bottleneck when first introduced to Australia). FA did change throughout the course of the nestling period (in both species), and some of the traits followed a similar trend as demonstrated in previous studies with other species. FA was also negatively correlated with nestling period for all traits but was not equal on the final day of measurement. Differences in these patterns were most likely due to the differing energetic costs of development, the functional importance of the trait, and other environmental stressors that might also influence FA in both species differently, perhaps masking potential bottleneck effects.

Comparisons of external body morphology between introduced NZ populations to their source population in the UK revealed overall changes in body size in the NZ populations of 4 species: Blackbirds, Greenfinches, and Song Thrushes decreased in size, while Redpolls increased in size compared to their UK counterparts. Within-species comparisons also revealed that all species exhibited some changes in trait morphology. The reasons for the rapid changes in body morphology in introduced NZ species is unknown, and although they may reflect adaptive change to the NZ environment, it is also possible they are a product of increased stress in the introduced range and as such may have implications for conservation programs

dealing with endangered species. The latter hypothesis was partly supported in my discovery of more deformities in NZ populations of introduced species than in the UK, although the significance of this relationship was only obtained when I used one species (Rook) that had a sample size of fewer than twenty individuals. Although these changes do not appear to be related to bottleneck size, there are a number of problems associated with obtaining unbiased estimates of abnormalities that mean it is difficult to rule out an effect of bottlenecks on increased rates of abnormalities and further study is warranted to determine if such a pattern is an early warning sign of DI in potentially inbred populations of endangered birds.

Despite finding a lack of correlations between changes in morphology and bottleneck severity, my results indicate that FA levels in wing traits (i.e., adult and nestling wing chord, P9, and ulna) appear to be the most reliable indicators of DI in potentially inbred populations. Selection for symmetry should be greater in wing traits due to their functional importance for survival (Møller and Nielsen 1997; Swaddle 1997; Swaddle and Witter 1997; Hovorka and Robertson 2000; Hambly et al. 2004), however, wing feathers are the most common area for asymmetry (Hambly et al. 2004) thus deviations from symmetry could reflect both genetic and environmental stresses on an individual since random perturbations could affect feathers at both the embryonic stage of development and during feather growth (Stige et al. 2005). Future studies of FA could focus primarily wing traits (i.e., FA in relation to structural composition; Aparicio and Bonal 2002) to examine for similarities in FA trends and patterns across taxa. For a conservation biologist interested in monitoring the stress that individuals in post-bottlenecked populations may be experiencing, my results suggest that FA in wing chord length or primary

length may be the best index, while traits based on osteological characters may be less useful.

A potential weakness of my study is the lack of genetic analyses to quantify the relationship between genetic diversity, FA and morphological changes, and to determine if the introduced birds of NZ display signs of genetic drift or decreased genetic variability. Throughout the thesis, I have assumed that species introduced in lower numbers have gone through a more severe bottleneck and as a result show less genetic diversity and higher levels of inbreeding. Further analyses are also needed to ascertain possible genetic changes in introduced species that might correlate with the observed morphological changes. Moreover, past analyses of genetic diversity in introduced birds in New Zealand (i.e., Ross 1983; Parkin and Cole 1985; Baker and Moeed 1987; Baker 1992; Merila et al. 1996) should be revised since improvements on genetic methods of evaluating diversity have since been made to increase their accuracy.

As most of the species introduced to NZ have also been introduced to other parts of the world (Long 1981), multi-location comparisons of FA levels and changes in morphology would be valuable in order to determine the effects of bottlenecks within the same species, as well as allow an examination of the possible differences in environmental and genetic stressors. Although a few studies have already been conducted examining potential genetic and morphological changes that have occurred in introduced species (e.g., Baker 1980; Baker and Mooed 1987) there is still a need to evaluate FA patterns since it is a measure that is commonly used to evaluate DI of a population (Parsons 1992; Palmer and Strobeck 1992; Lens et al. 2002). Meta analyses using other indicators of fitness, such as hatching failure (e.g., Briskie and Mackintosh 2004) and changes in immunocompetence and parasite loads (e.g., Allen

2008, unpubl. data) could also be performed to assess fitness and DI of introduced populations in NZ and would further support any correlations with bottleneck effects. Moreover, Lens and Eggermont (2008) proposed to combine studies of ptilochronology (see Grubb 1989, 2006) and FA to better evaluate anthropogenic stress. As I currently have data on the light and dark growth-bar patterns of tail feathers for both my NZ and UK populations of study species, I plan on doing further analyses on ptilochronology, incorporating my findings from this study.

In summary, I found that FA appeared to be related to bottleneck size for only some external traits, however osteological characters, morphometrics, and abnormalities did not appear to be related to bottleneck size. Nevertheless, higher levels of FA in certain traits, in particular those of the wing, may be an indicator of DI in some species, thus further examination is warranted. Calculating FA levels and differences in morphology is not difficult (Palmer and Strobeck 2003). Moreover, their relative ease of use and, in most cases, non-invasive methods, could strengthen findings from other fitness studies such as hatching failure or immunocompetence, and thus would make it a practical tool in species conservation, especially when dealing with endangered species.

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APPENDICES

The following appendices A and B contain the FA and ME scores for chapter 1 and 2, respectively. All scores were calculated according to Palmer and Strobeck (2003)[1] and their formulas and definitions are as follows:

FA1: mean [R – L], the absolute unsigned asymmetry in mm

ME1: 0.798 √ MS_{err}, mean difference between repeated measurements on one side (in mm), where MS_{err} is the mean squares of the variance of repeated measurements from the two-way mixed model ANOVA.

FA10a: 0.798 √ (MS_{si} – MS_{err}), the estimated underlying DI variance (in mm), where MS_{si} is the mean squares of the sides × individuals interaction and MS_{err} is the mean squares of the variance of repeated measurements from the two-way mixed model ANOVA.

FA4a: 0.798 √ MS_{si}, the estimated between sides variation between two samples (in mm) that factors out directional asymmetry, where MS_{si} is the mean squares of the sides × individuals interaction from the two-way mixed model ANOVA.

FA8a: mean [ln R / L], the relative asymmetry (in mm) expressed as a ratio between the sides.

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[1] Palmer, A. R., and C. Strobeck. 2003. Fluctuating Asymmetry Analyses Revisited. In: M. Polak (ed.) Developmental Instability: its causes and consequences, pp. 279-319. Oxford University Press, Oxford.

Appendix A

Appendix A.1a. FA and ME scores of bill length in after hatch year (AHY) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	AHY	F	9	0.1341	0.0940	0.1152	0.1695	0.0089
T. merula	UK			11	0.1085	0.0842	0.0867	0.1355	0.0068
Chaffinch	NZ			12	0.1008	0.0816	0.0950	0.1421	0.0108
F. coelebs	UK			8	0.0938	0.0697	0.1153	0.1575	0.0099
Goldfinch	NZ			10	0.0897	0.0718	0.0670	0.1091	0.0088
C. carduelis									
Redpoll	NZ			6	0.1883	0.1168	0.1005	0.1697	0.0275
C. flammea	UK			2	0.0750	0.0830	0.0506	0.1036	0.0114
Yellowhammer	NZ			7	0.1229	0.0855	0.0495	0.1048	0.0156
E. citrinella									
Blackbird	NZ	AHY	M	9	0.1767	0.1119	0.1480	0.2131	0.0113
T. merula	UK			11	0.1082	0.0715	0.1049	0.1471	0.0068
Chaffinch	NZ			28	0.1130	0.0804	0.0906	0.1370	0.0117
F. coelebs	UK			8	0.1550	0.0718	0.1058	0.1481	0.0158
Goldfinch	NZ			7	0.1048	0.0809	0.0696	0.1176	0.0094
C. carduelis									
Greenfinch	UK			4	0.0908	0.0792	0.0148	0.0812	0.0080
C. chloris									
Redpoll	NZ			14	0.1188	0.0879	0.0736	0.1259	0.0166
C. flammea									
Yellowhammer	NZ			10	0.1433	0.0940	0.0700	0.1272	0.0176
E. citrinella									
Dunnock	NZ	AHY	UNK	7	0.1500	0.0870	0.1235	0.1745	0.0204
P. modularis	UK			20	0.1242	0.0903	0.1239	0.1766	0.0171
Redpoll	NZ			9	0.2167	0.0862	0.1662	0.2210	0.0306
C. flammea									
Song Thrush	NZ			6	0.1894	0.0802	0.1394	0.1887	0.0149
T. philomelos	UK			4	0.1183	0.0980	0.0821	0.1404	0.0094

Appendix A.1b. FA and ME scores of bill length in hatch year (HY) and unknown aged (UNK) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	HY	F	9	0.1022	0.0937	0.0777	0.1336	0.0066
T. merula	UK			19	0.1430	0.0900	0.1261	0.1788	0.0095
Chaffinch	NZ			28	0.1169	0.0757	0.0986	0.1426	0.0123
F. coelebs	UK			18	0.1583	0.1074	0.1354	0.1976	0.0170
Goldfinch	UK			4	0.1275	0.0767	0.0886	0.1329	0.0126
C. carduelis									
Greenfinch	NZ			22	0.0945	0.0780	0.0683	0.1143	0.0090
C. chloris	UK			22	0.1174	0.0784	0.0854	0.1307	0.0111
House Sparrow	NZ			16	0.0956	0.0648	0.0794	0.1169	0.0100
P. domesticus	UK			2	0.0950	0.0609	0.0305	0.0714	0.0100
Yellowhammer	NZ			6	0.0972	0.0876	0.1895	0.2481	0.0123
E. citrinella									
Blackbird	NZ	HY	M	9	0.1722	0.0808	0.1074	0.1544	0.0109
T. merula	UK			23	0.1442	0.0811	0.1272	0.1757	0.0095
Chaffinch	NZ			30	0.0944	0.0714	0.0751	0.1164	0.0098
F. coelebs	UK			6	0.0872	0.0992	0.0408	0.1111	0.0091
Goldfinch	NZ			6	0.1639	0.0561	0.0490	0.0822	0.0155
C. carduelis									
Greenfinch	NZ			22	0.0811	0.0616	0.0688	0.1044	0.0075
C. chloris	UK			33	0.1141	0.0731	0.0947	0.1371	0.0109
House Sparrow	NZ			15	0.1280	0.0797	0.0766	0.1231	0.0134
P. domesticus	UK			3	0.1578	0.1107	0.1355	0.1994	0.0173
Redpoll	NZ			5	0.1780	0.1164	0.1587	0.2266	0.0243
C. flammea	UK			22	0.1550	0.0854	0.0690	0.1202	0.0216
Yellowhammer	NZ			13	0.1164	0.1000	0.0768	0.1372	0.0146
E. citrinella									
Dunnock	NZ	HY	UNK	35	0.1495	0.0859	0.1444	0.1966	0.0210
P. modularis	UK			87	0.1211	0.0931	0.0975	0.1514	0.0166
Goldfinch	NZ			36	0.1268	0.0764	0.1331	0.1801	0.0127
C. carduelis	UK			63	0.1110	0.0663	0.0782	0.1165	0.0113
Redpoll	NZ			12	0.1325	0.1032	0.1042	0.1641	0.0186
C. flammea	UK			20	0.1352	0.0921	0.0932	0.1466	0.0200
Song Thrush	NZ			17	0.1345	0.0908	0.1165	0.1691	0.0109
T. philomelos	UK			47	0.1340	0.0860	0.0982	0.1478	0.0108
House Sparrow	NZ	UNK	F	38	0.0916	0.0698	0.0708	0.1113	0.0098
P. domesticus	UK			7	0.1362	0.0802	0.0758	0.1226	0.0143
House Sparrow	NZ	UNK	M	39	0.1370	0.0691	0.1146	0.1565	0.0145
P. domesticus	UK			7	0.1067	0.0618	0.0714	0.1070	0.0113

Appendix A.2a. FA and ME scores of the tarsus length in after hatch year (AHY) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	AHY	F	10	0.2607	0.0924	0.2619	0.3338	0.0070
T. merula	UK			10	0.1800	0.1005	0.1565	0.2164	0.0048
Chaffinch	NZ			12	0.1272	0.1092	0.1174	0.1805	0.0063
F. coelebs	UK			8	0.1679	0.0800	0.1469	0.1969	0.0084
Goldfinch *C. carduelis*	NZ			10	0.2273	0.0739	0.1322	0.1780	0.0137
Redpoll *C. flammea*	NZ			6	0.1517	0.1082	0.1454	0.2084	0.0097
Yellowhammer *E. citrinella*	NZ			8	0.2008	0.0907	0.2220	0.2866	0.0095
Blackbird	NZ	AHY	M	9	0.3256	0.1292	0.3157	0.4077	0.0086
T. merula	UK			11	0.2512	0.1181	0.2456	0.3232	0.0065
Chaffinch	NZ			28	0.1261	0.0849	0.1032	0.1522	0.0061
F. coelebs	UK			6	0.1683	0.0900	0.1332	0.1863	0.0081
Goldfinch *C. carduelis*	NZ			7	0.2467	0.0902	0.2269	0.2921	0.0148
Greenfinch *C. chloris*	NZ			6	0.0978	0.0671	0.0848	0.1237	0.0049
Redpoll *C. flammea*	NZ			14	0.1886	0.1019	0.1570	0.2176	0.0120
Yellowhammer *E. citrinella*	NZ			9	0.1996	0.0852	0.1138	0.1633	0.0091
Dunnock	NZ	AHY	UNK	6	0.1567	0.0768	0.1437	0.1921	0.0066
P. modularis	UK			20	0.2278	0.0804	0.2218	0.2833	0.0095
Redpoll *C. flammea*	NZ			8	0.1675	0.0995	0.1102	0.1677	0.0111
Song Thrush	NZ			6	0.2300	0.0656	0.1929	0.2452	0.0064
T. philomelos	UK			4	0.1183	0.1003	0.0724	0.1339	0.0032

Appendix A.2b. FA and ME scores of the tarsus length in hatch year (HY) and unknown aged (UNK) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	HY	F	8	0.2371	0.1352	0.2352	0.3182	0.0062
T. merula	UK			21	0.2337	0.0968	0.2379	0.3070	0.0061
Chaffinch	NZ			27	0.1681	0.1036	0.1598	0.2214	0.0083
F. coelebs	UK			18	0.1478	0.0848	0.1223	0.1722	0.0072
Goldfinch	NZ			2	0.1800	0.1111	0.0457	0.1244	0.0111
C. carduelis	UK			4	0.2875	0.0838	0.2303	0.2943	0.0176
Greenfinch	NZ			21	0.1225	0.0838	0.1002	0.1487	0.0060
C. chloris	UK			22	0.1574	0.0725	0.1160	0.1595	0.0077
House Sparrow	NZ			17	0.1775	0.1136	0.1559	0.2222	0.0080
P. domesticus	UK			2	0.1733	0.0900	0.1813	0.2396	0.0084
Yellowhammer	NZ			7	0.1114	0.0967	0.0697	0.1290	0.0051
E. citrinella									
Blackbird	NZ	HY	M	9	0.2215	0.1490	0.1726	0.2586	0.0058
T. merula	UK			24	0.2378	0.1094	0.2042	0.2730	0.0062
Chaffinch	NZ			29	0.2032	0.0942	0.1786	0.2382	0.0097
F. coelebs	UK			6	0.1133	0.0829	0.0924	0.1403	0.0055
Goldfinch	NZ			6	0.1772	0.0973	0.1318	0.1885	0.0106
C. carduelis	UK			5	0.1193	0.0648	0.1106	0.1502	0.0071
Greenfinch	NZ			22	0.1845	0.1034	0.1477	0.2083	0.0090
C. chloris	UK			32	0.1825	0.0765	0.1647	0.2157	0.0090
House Sparrow	NZ			15	0.1960	0.0998	0.1266	0.1844	0.0088
P. domesticus	UK			2	0.1433	0.0975	0.1408	0.1981	0.0066
Redpoll	NZ			4	0.2392	0.1116	0.2317	0.3049	0.0154
C. flammea	UK			22	0.2598	0.0867	0.1740	0.2301	0.0167
Yellowhammer	NZ			11	0.1715	0.0249	0.1845	0.2273	0.0078
E. citrinella									
Dunnock	NZ	HY	UNK	34	0.2001	0.1105	0.1853	0.2523	0.0085
P. modularis	UK			88	0.1739	0.1071	0.1492	0.2118	0.0073
Goldfinch	NZ			35	0.1861	0.0954	0.1783	0.2383	0.0111
C. carduelis	UK			63	0.1766	0.0803	0.1550	0.2061	0.0107
Redpoll	NZ			11	0.0736	0.0996	0.0085	0.1001	0.0048
C. flammea	UK			20	0.2610	0.0930	0.2013	0.2635	0.0168
Song Thrush	NZ			17	0.2069	0.1336	0.1877	0.2659	0.0058
T. philomelos	UK			46	0.2081	0.0905	0.2162	0.2799	0.0058
House Sparrow	NZ	UNK	F	39	0.2036	0.1019	0.1461	0.2059	0.0093
P. domesticus	UK			7	0.1181	0.0867	0.0966	0.1466	0.0053
House Sparrow	NZ	UNK	M	39	0.1979	0.0929	0.1895	0.2500	0.0089
P. domesticus	UK			8	0.2404	0.0739	0.1718	0.2230	0.0110

Appendix A.3a. FA and ME scores of the wing chord in after hatch year (AHY) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	AHY	F	9	1.1667	0.2549	1.0605	1.3236	0.0095
T. merula	UK			10	0.6300	0.2294	0.5833	0.7503	0.0051
Chaffinch	NZ			13	0.4872	0.2096	0.4685	0.6109	0.0061
F. coelebs	UK			10	0.3833	0.2185	0.2950	0.4223	0.0048
Goldfinch *C. carduelis*	NZ			9	0.6111	0.1801	0.3467	0.4612	0.0083
Greenfinch *C. chloris*	NZ			2	1.2500	0.1995	1.4013	1.7278	0.0149
Redpoll *C. flammea*	NZ			5	0.5000	0.1262	0.4958	0.6202	0.0075
Yellowhammer *E. citrinella*	NZ			7	0.3333	0.2042	0.2215	0.3395	0.0041
Blackbird	NZ	AHY	M	11	1.2121	0.2549	1.1046	1.3766	0.0096
T. merula	UK			11	0.5758	0.3142	0.4429	0.6268	0.0045
Chaffinch	NZ			28	0.3810	0.2385	0.3644	0.5060	0.0044
F. coelebs	UK			6	0.3889	0.2206	0.3672	0.5009	0.0045
Goldfinch *C. carduelis*	NZ			10	0.5167	0.2124	0.5843	0.7465	0.0067
Greenfinch	NZ			7	0.8095	0.7020	0.6082	1.0235	0.0096
C. chloris	UK			6	0.3889	0.1330	0.4148	0.5251	0.0045
House Sparrow *P. domesticus*	NZ			5	0.8333	0.2060	0.9185	1.1437	0.0109
Redpoll *C. flammea*	NZ			15	0.4111	0.1574	0.4028	0.5178	0.0059
Yellowhammer *E. citrinella*	NZ			9	0.5370	0.2032	0.5440	0.6966	0.0065
Dunnock	NZ	AHY	UNK	8	0.3750	0.2076	0.2759	0.3966	0.0055
P. modularis	UK			18	0.5556	0.1801	0.4911	0.6278	0.0081
Redpoll *C. flammea*	NZ			9	0.5370	0.2488	0.2778	0.4215	0.0080
Song Thrush	NZ			5	0.6000	0.2303	0.1781	0.3172	0.0052
T. philomelos	UK			5	0.5667	0.2060	0.6807	0.8587	0.0049

Appendix A.3b. FA and ME scores of the wing chord in hatch year (HY) and unknown (UNK) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	HY	F	11	0.3333	0.2406	0.2863	0.4253	0.0028
T. merula	UK			24	0.4167	0.2230	0.3909	0.5281	0.0034
Chaffinch	NZ			31	0.4247	0.2266	0.4488	0.5945	0.0053
F. coelebs	UK			18	0.3889	0.1674	0.3760	0.4900	0.0049
Goldfinch	UK			9	0.3519	0.2172	0.2238	0.3497	0.0047
C. carduelis									
Greenfinch	NZ			21	0.4365	0.4053	0.1923	0.4687	0.0052
C. chloris	UK			25	0.4333	0.2111	0.3871	0.5190	0.0052
House Sparrow	NZ			16	0.5417	0.2076	0.5086	0.6566	0.0074
P. domesticus	UK			2	0.7500	0.1629	0.8360	1.0368	0.0107
Yellowhammer	NZ			6	0.4167	0.1629	0.2509	0.3478	0.0050
E. citrinella									
Blackbird	NZ	HY	M	9	0.4074	0.2367	0.3953	0.5389	0.0033
T. merula	UK			24	0.6250	0.2659	0.5950	0.7757	0.0049
Chaffinch	NZ			30	0.3944	0.1904	0.4006	0.5263	0.0046
F. coelebs	UK			5	0.4000	0.2416	0.3394	0.4808	0.0048
Goldfinch	NZ			5	0.3667	0.2523	0.3091	0.4549	0.0049
C. carduelis	UK			8	0.2500	0.2076	0.0814	0.2303	0.0033
Greenfinch	NZ			20	0.4167	0.1857	0.4306	0.5592	0.0049
C. chloris	UK			37	0.4595	0.2224	0.3266	0.4577	0.0053
House Sparrow	NZ			15	0.7333	0.2061	0.5923	0.7541	0.0102
P. domesticus	UK			3	1.3889	0.1629	1.8009	2.2116	0.0186
Redpoll	NZ			6	0.3333	0.2206	0.2234	0.3515	0.0049
C. flammea	UK			23	0.4130	0.2519	0.1991	0.3506	0.0060
Yellowhammer	NZ			13	0.5897	0.2304	0.4729	0.6233	0.0069
E. citrinella									
Dunnock	NZ	HY	UNK	34	0.4127	0.2260	0.3632	0.4990	0.0061
P. modularis	UK			91	0.4377	0.2323	0.3381	0.4748	0.0065
Goldfinch	NZ			33	0.2828	0.1771	0.2621	0.3666	0.0037
C. carduelis	UK			65	0.3897	0.2157	0.2303	0.3551	0.0051
Redpoll	NZ			10	0.3500	0.2124	0.2314	0.3542	0.0052
C. flammea	UK			21	0.4286	0.2162	0.2777	0.4030	0.0063
Song Thrush	NZ			15	0.4222	0.1457	0.3377	0.4385	0.0037
T. philomelos	UK			49	0.5952	0.2741	0.4975	0.6681	0.0052
House Sparrow	NZ	UNK	F	40	0.4500	0.2076	0.4007	0.5329	0.0061
P. domesticus	UK			7	0.7143	0.2042	0.6005	0.7633	0.0097
House Sparrow	NZ	UNK	M	40	0.3625	0.1910	0.3848	0.5085	0.0048
P. domesticus	UK			9	0.3519	0.1958	0.3048	0.4215	0.0046

Appendix A.4a. FA and ME scores of P9 length in after hatch year (AHY) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	AHY	F	6	0.5278	0.1487	0.4929	0.6217	0.0065
T. merula	UK			8	0.5208	0.2573	0.4278	0.5837	0.0063
Chaffinch	NZ			10	0.5333	0.1995	0.5375	0.6879	0.0093
F. coelebs	UK			9	0.6111	0.2366	0.3850	0.5275	0.0106
Goldfinch *C. carduelis*	NZ			4	0.9583	0.1629	0.5937	0.7452	0.0175
Redpoll *C. flammea*	NZ			3	0.4444	0.2304	0.3842	0.5239	0.0087
Yellowhammer *E. citrinella*	NZ			7	0.5238	0.2888	0.4858	0.6614	0.0086
Blackbird	NZ	AHY	M	8	0.5208	0.2764	0.4105	0.5738	0.0063
T. merula	UK			8	0.4583	0.2155	0.4694	0.6140	0.0055
Chaffinch *F. coelebs*	NZ			24	0.3750	0.2328	0.3151	0.4507	0.0060
Goldfinch *C. carduelis*	NZ			7	0.6667	0.2536	0.4327	0.5875	0.0118
Greenfinch *C. chloris*	NZ			6	0.4167	0.2741	0.1090	0.3049	0.0066
House Sparrow *P. domesticus*	NZ			5	0.4333	0.2185	0.2266	0.3533	0.0078
Redpoll *C. flammea*	NZ			8	0.5208	0.1910	0.3870	0.5110	0.0098
Yellowhammer *E. citrinella*	NZ			9	0.9074	0.2239	0.7884	0.9913	0.0143
Dunnock	NZ	AHY	UNK	8	0.5417	0.2510	0.3871	0.5365	0.0119
P. modularis	UK			13	0.5000	0.2096	0.5176	0.6677	0.0109
Redpoll *C. flammea*	NZ			7	0.7381	0.3587	0.4147	0.6217	0.0151
Song Thrush	NZ			4	0.5000	0.1821	0.6060	0.7642	0.0059
T. philomelos	UK			2	0.5000	0.2821	0.5151	0.6911	0.0061

Appendix A.4b. FA and ME scores of P9 length in hatch year (HY) and unknown (UNK) NZ and UK populations.

Species	Location	Age	Sex	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	HY	F	11	0.4848	0.2141	0.4317	0.5704	0.0061
T. merula	UK			24	0.4931	0.2155	0.4361	0.5760	0.0061
Chaffinch	NZ			31	0.4516	0.2810	0.3373	0.4996	0.0079
F. coelebs	UK			19	0.3860	0.2114	0.3263	0.4521	0.0069
Greenfinch	NZ			21	0.5556	0.2220	0.3543	0.4874	0.0089
C. chloris	UK			25	0.4933	0.2061	0.3167	0.4393	0.0080
House Sparrow *P. domesticus*	NZ			15	0.4444	0.1927	0.2933	0.4077	0.0085
Yellowhammer *E. citrinella*	NZ			6	0.3611	0.2659	0.3581	0.5128	0.0060
Blackbird	NZ	HY	M	8	0.7917	0.2764	0.7412	0.9489	0.0097
T. merula	UK			23	0.6884	0.7819	0.1819	0.8130	0.0083
Chaffinch	NZ			30	0.4556	0.2361	0.2821	0.4185	0.0075
F. coelebs	UK			5	0.3333	0.2416	0.0724	0.2573	0.0055
Goldfinch	NZ			4	1.0417	0.1152	0.5278	0.6566	0.0184
C. carduelis	UK			7	0.8095	0.3794	0.1748	0.4356	0.0143
Greenfinch	NZ			20	0.6667	0.2155	0.3503	0.4801	0.0105
C. chloris	UK			36	0.4815	0.2335	0.4107	0.5546	0.0076
House Sparrow	NZ			13	0.3846	0.1629	0.3168	0.4208	0.0074
P. domesticus	UK			3	0.5000	0.1330	0.4477	0.5643	0.0093
Redpoll	NZ			6	0.6667	0.1629	0.6247	0.7823	0.0127
C. flammea	UK			23	0.6884	0.2402	0.1575	0.3080	0.0133
Yellowhammer *E. citrinella*	NZ			13	0.4103	0.3192	0.3743	0.5586	0.0067
Dunnock	NZ	HY	UNK	34	0.3725	0.2091	0.2967	0.4192	0.0083
P. modularis	UK			92	0.4656	0.2259	0.3870	0.5251	0.0105
Goldfinch	NZ			32	0.5417	0.2286	0.3686	0.5060	0.0095
C. carduelis	UK			64	0.6536	0.2401	0.3420	0.4828	0.0117
Redpoll	NZ			11	0.6515	0.1965	0.2242	0.3376	0.0128
C. flammea	UK			20	0.7500	0.1927	0.3346	0.4528	0.0147
Song Thrush	NZ			15	0.6111	0.5797	0.4054	0.7633	0.0077
T. philomelos	UK			48	0.7674	0.3241	0.8288	1.0656	0.0095
House Sparrow	NZ	UNK	F	22	0.4242	0.2196	0.3298	0.4598	0.0080
P. domesticus	UK			4	0.1667	0.1411	0.1273	0.2103	0.0031
House Sparrow *P. domesticus*	NZ	UNK	M	22	0.3636	0.2251	0.1680	0.3049	0.0067

Appendix B

Appendix B.1. FA and ME scores of the femur length in NZ and UK populations.

Species	Location	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	13	0.1292	0.0321	0.1212	0.1519	0.0044
T. merula	UK	13	0.1113	0.0412	0.1001	0.1293	0.0039
Cirl Bunting *E. cirlus*	NZ	9	0.0522	0.0206	0.0518	0.0667	0.0030
Dunnock	NZ	6	0.1006	0.0200	0.1008	0.1250	0.0055
P. modularis	UK	10	0.0667	0.0218	0.0585	0.0749	0.0037
Greenfinch	NZ	14	0.0393	0.0187	0.0256	0.0365	0.0022
C. chloris	UK	11	0.0485	0.0261	0.0519	0.0687	0.0028
House Sparrow	NZ	13	0.1051	0.0166	0.0822	0.1020	0.0057
P. domesticus	UK	14	0.0769	0.0344	0.0758	0.0990	0.0043
Rook	NZ	12	0.2233	0.0737	0.2096	0.2671	0.0046
C. frugilegus	UK	10	0.1967	0.0398	0.1750	0.2180	0.0040
Yellowhammer	NZ	13	0.0462	0.0291	0.0426	0.0597	0.0026
E. citrinella	UK	12	0.0528	0.0191	0.0497	0.0638	0.0029

Appendix B.2. FA and ME scores of the humerus length in NZ and UK populations.

Species	Location	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	14	0.1457	0.0368	0.0875	0.1133	0.0049
T. merula	UK	12	0.1083	0.0405	0.1078	0.1381	0.0037
Cirl Bunting *E. cirlus*	NZ	9	0.0622	0.0211	0.0362	0.0491	0.0033
House Sparrow	NZ	13	0.0913	0.0467	0.0781	0.1065	0.0050
P. domesticus	UK	12	0.0878	0.0340	0.0981	0.1249	0.0049
Rook	NZ	13	0.2249	0.1075	0.1909	0.2573	0.0036
C. frugilegus	UK	11	0.2930	0.0643	0.2659	0.3319	0.0047
Yellowhammer	NZ	11	0.0488	0.0366	0.0341	0.0555	0.0025
E. citrinella	UK	11	0.1055	0.0330	0.0833	0.1072	0.0055

Appendix B.3. FA and ME scores of the radius length in NZ and UK populations.

Species	Location	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	10	0.1431	0.0593	0.1226	0.1614	0.0046
T. merula	UK	14	0.1600	0.0610	0.1139	0.1523	0.0052
Cirl Bunting	NZ	9	0.0671	0.0417	0.0633	0.0880	0.0035
E. cirlus							
House Sparrow	NZ	11	0.1136	0.0506	0.0751	0.1050	0.0062
P. domesticus	UK	13	0.1303	0.0634	0.1123	0.1515	0.0073
Rook	NZ	10	0.2860	0.0831	0.3160	0.3958	0.0041
C. frugilegus	UK	11	0.3945	0.0632	0.2119	0.2671	0.0055
Yellowhammer	NZ	9	0.1296	0.0316	0.1494	0.1857	0.0060
E. citrinella	UK	14	0.0588	0.0425	0.0525	0.0771	0.0029

Appendix B.4. FA and ME scores of the ulna length in NZ and UK populations.

Species	Location	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	13	0.1283	0.0298	0.1482	0.1840	0.0037
T. merula	UK	15	0.1507	0.0341	0.2182	0.2694	0.0043
Cirl Bunting	NZ	7	0.0644	0.0171	0.0333	0.0442	0.0029
E. cirlus							
Dunnock	NZ	7	0.0967	0.0292	0.0935	0.1182	0.0053
P. modularis	UK	11	0.0385	0.0240	0.0348	0.0489	0.0020
Greenfinch	NZ	12	0.0550	0.0338	0.0400	0.0595	0.0021
C. chloris	UK	11	0.0727	0.0330	0.0577	0.0780	0.0031
House Sparrow	NZ	11	0.0542	0.0249	0.0488	0.0647	0.0026
P. domesticus	UK	12	0.1119	0.0406	0.0809	0.1071	0.0055
Rook	NZ	9	0.4293	0.0617	0.3979	0.4913	0.0055
C. frugilegus	UK	10	0.3377	0.0468	0.2675	0.3310	0.0043
Yellowhammer	NZ	10	0.0980	0.0237	0.0730	0.0925	0.0040
E. citrinella	UK	13	0.0918	0.0428	0.0704	0.0963	0.0040

Appendix B.5. FA and ME scores of the tibia length in NZ and UK populations.

Species	Location	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	13	0.1551	0.0519	0.1442	0.1841	0.0032
T. merula	UK	10	0.1757	0.0428	0.1754	0.2191	0.0037
Cirl Bunting *E. cirlus*	NZ	9	0.1015	0.0283	0.0860	0.1090	0.0037
Greenfinch	NZ	12	0.0811	0.0570	0.0764	0.1096	0.0028
C. chloris	UK	10	0.1037	0.0577	0.0918	0.1264	0.0037
House Sparrow	NZ	12	0.1183	0.0588	0.1220	0.1606	0.0041
P. domesticus	UK	14	0.1348	0.0491	0.0619	0.0903	0.0046
Rook	NZ	11	0.2385	0.1390	0.2461	0.3319	0.0028
C. frugilegus	UK	11	0.2000	0.0792	0.1999	0.2573	0.0024
Yellowhammer	NZ	13	0.0754	0.0463	0.0695	0.0969	0.0026
E. citrinella	UK	8	0.0946	0.0633	0.0726	0.1092	0.0033

Appendix B.6. FA and ME scores of the tarsometatarsus length in NZ and UK populations.

Species	Location	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	11	0.1112	0.0199	0.1184	0.1463	0.0034
T. merula	UK	6	0.3794	0.0280	0.1525	0.1889	0.0116
Cirl Bunting *E. cirlus*	NZ	10	0.0467	0.0195	0.0287	0.0402	0.0026
Dunnock	NZ	5	0.1593	0.0210	0.1000	0.1242	0.0075
P. modularis	UK	9	0.1270	0.0443	0.0895	0.1183	0.0060
Greenfinch	NZ	12	0.0703	0.0307	0.0597	0.0794	0.0039
C. chloris	UK	6	0.2272	0.0544	0.0786	0.1106	0.0125
House Sparrow	NZ	11	0.1021	0.0264	0.0891	0.1123	0.0054
P. domesticus	UK	10	0.2627	0.0708	0.2230	0.2821	0.0141
Rook	NZ	8	0.2333	0.0652	0.2415	0.3028	0.0044
C. frugilegus	UK	8	0.2167	0.0369	0.2208	0.2730	0.0040
Yellowhammer	NZ	11	0.1636	0.0227	0.0962	0.1200	0.0083
E. citrinella	UK	9	0.1670	0.0579	0.1298	0.1692	0.0087

Appendix B.7. FA and ME scores of the sternum length in NZ and UK populations.

Species	Location	N	FA1	ME1	FA10a	FA4a	FA8a
Blackbird	NZ	16	0.2258	0.0371	0.2246	0.2776	0.0184
T. merula	UK	6	0.2111	0.0444	0.2556	0.3162	0.0170
Cirl Bunting	NZ	6	0.2111	0.0474	0.1564	0.1973	0.0261
E. cirlus							
Dunnock	NZ	6	0.1944	0.0275	0.1855	0.2288	0.0249
P. modularis	UK	9	0.2407	0.0724	0.2365	0.2986	0.0319
Greenfinch	NZ	13	0.2215	0.0274	0.2217	0.2730	0.0221
C. chloris	UK	9	0.4215	0.0576	0.2949	0.3657	0.0419
House Sparrow	NZ	12	0.3764	0.0427	0.3050	0.3760	0.0397
P. domesticus	UK	14	0.3329	0.0648	0.2674	0.3338	0.0354
Rook	NZ	10	0.7077	0.0453	0.6296	0.7725	0.0237
C. frugilegus	UK	14	0.5490	0.0334	0.2966	0.3648	0.0190
Yellowhammer	NZ	13	0.1815	0.0251	0.1668	0.2058	0.0192
E. citrinella	UK	14	0.1788	0.0415	0.1733	0.2163	0.0194